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Vitamin B12 requirement of the weanling pig

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VITAMIN B_{12} REQUIREMENT OF THE WEANLING PIG

by

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INTRODUCTION

Animal protein has long been recognized as being superior to plant protein in the feeding of swine and poultry. Reproduction, lactation and growing-fattening results have proved this in experimental work and also in general feeding of non ruminants on the farm.

Since the amino acid content of some plant proteins, especially soybean oil meal, generally compares favorably with the amino acid content of some animal proteins, the advantage in feeding animal protein could not be satisfactorily explained on purely a protein content basis. There are additional factors, but it is now known that the superiority of animal protein is largely due to its vitamin B_{12} content.

We are faced with the fact that protein supplements from by-products of the meat packing industry are not as efficient as they were several years ago. This may be explained by the increased ability of industry to extract vitamins, hormones, and other nutrients from glands and tissues for the pharmaceutical trade. Therefore, many of these important nutrients are being removed which formerly went into the by-products to be used as animal food. Much of the better by-products are also channeled into the dog food industry which has now reached a volume of several million dollars per year.

There are indications that vitamin B_{12} has a number of functions in the living organism. As yet, little is known about its mode of action. In due time, the many observations made and additional experimental work will doubtless be welded together into a coherent theory on the functions of vitamin B_{12}.

Vitamin B_{12} has proved its value in supplementing vegetable proteins in swine rations. This study was made to determine the nutritional requirement of the weanling pig for vitamin B_{12}.

REVIEW OF LITERATURE

Comparison of Protein Supplements

The fact that various protein supplements vary in value as a supplement in swine feeding has long been recognized. In 1907, Kennedy and Robbins (114) at the Iowa Agricultural Experiment Station found that vegetable protein rations were inferior to rations supplemented with animal protein. In 1917, Ashbrook (13) stated that fish meal was superior to tankage in all tests when compared as a supplement. In 1921, Robison (173) and Morrison and Bohstedt (139) obtained similar results when fish meal was compared with meat meal or tankage in drylot. When vegetable proteins were compared with animal proteins, the growth rate was even less.

In 1943, Robison (174) found that pigs fed soybean oil meal on pasture made faster gains than similar pigs fed tankage. In drylot the addition of soybean oil meal produced slower gains in pigs than tankage and linseed meal. A greater number of those on the soybean oil meal ration became unthrifty thus suggesting a nutritional deficiency in the ration. Fish meal was effective in correcting the deficiency. Tankage was less effective but of some benefit. Krider et al. (115) obtained similar results when the pigs had access to rye pasture during the nursing period. After transfer to drylot, they gained satisfactorily to 75 pounds. Continuous drylot feeding of vegetable proteins did not produce satisfactory gains. Fortification of the ration with either six B-vitamins, 6 per cent dried corn distillers solubles or 10 per cent alfalfa meal failed to correct this all plant ration deficiency. The addition of fish meal and tankage and six B-vitamins produced the most rapid gains. Fairbanks et al. (81) also obtained very similar results on pasture.

Catron et al. (48) used a basal ration of corn, soybean oil meal, minerals and vitamins A and D to compare various fish products for growing-fattening pigs. This basal ration was improved by addition of condensed fish solubles, fish meal, meat scraps and semi-solid fish. The results indicate that 2 per cent condensed fish solubles is equivalent to 4 per cent fish meal or 5 per cent meat scraps in supplementing the basal ration. Lepley (119) obtained similar results. He also found that a corn soybean oil meal ration supplemented with a vitamin B_{12} concentrate gave gains equally as good as fish meal, meat scraps or condensed fish solubles.

In five tests, Krider and Terrill (116) found a basal ration of corn, soybean oil meal, 6 per cent meat scraps, minerals and vitamins A and D to be inadequate for pigs kept in drylot from birth. This ration was improved by addition of either alfalfa meal, condensed sardine solubles, menhaden fish solubles or dried corn distiller's solubles. Increased gains were attributed to the content of the various water soluble vitamins present in the various products. Geurin et al. (86) obtained similar results with condensed fish solubles and liquid fish. He concluded that fish solubles contain dietary factors other than protein which greatly improve a corn-soybean oil meal ration for growing fattening pigs.

The amount of protein in condensed fish solubles is small and Almquist (3,4) and Block and Mitchell (29, 30) have presented evidence to show that the quality of protein is poor. This is further evidence that condensed fish solubles contain factors other than protein which are beneficial for growing-fattening swine. It is now believed that vitamin B_{12} is the factor largely responsible for the high feeding value of condensed fish solubles.

History and Isolation of Vitamin B_{12}

The observation was made that built-up floor litter provided the dietary factors including the unidentified animal protein or vitamin factors necessary for the production of eggs of maximum hatchability (113). This unidentified factor was also found to be a growth promoting factor (26, 27, 91, 92, 132, 149). Extensive work with cow manure has shown that it contains a chick growth factor (124, 177, 178, 179, 180). Since the isolation and crystallization of the anti-pernicious anemia factor or vitamin B_{12} from liver (169, 198), work has been done in which the growth response of chicks to crystalline vitamin B_{12}, liver extract and acid precipitate of water extract of cow manure was essentially the same (124). Over one hundred organisms isolated from poultry house litter and droppings have shown vitamin B_{12} producing ability (90).

Functions and Relationships of Vitamin B_{12}

A growth promoting action of vitamin B_{12} has been clearly demonstrated in pigs and chicks. There is also a suggestion that it may in some instances augment the growth of backward children (221). It is of great value in treating pernicious anemia (50, 68, 201, 202, 220). Traina (215) found that vitamin B_{12} has an anti-anaphylactic effect but this has not been confirmed by other workers.

It has been shown that vitamin B_{12} is concerned in transmethylation reactions (19, 87, 183, 187). Thus chicks and rats on a deficient diet can utilize homocystine as an alternative to methionine only when vitamin B_{12} is given. Dubnoff (72) has shown that vitamin B_{12} augments the reduction of S-S compounds in liver slices. Vitamin B_{12} reduced blood levels of non-protein nitrogen. It appears to function in metabolism by enhancing utilization of circulating amino acids for building tissues (1, 37, 51, 53, 67, 133, 136, 152, 165, 166, 176, 182, 183, 203, 204, 205, 207, 211). Aschkenasy (12) thinks that vitamin B_{12} contains two different fractions, one of which (probably dimethylbenziminazole) is anabolic and can be substituted by methionine, the other (probably the cobalt containing one) being only hematopoietic.

A definite peak of embryonic mortality occurred on the seventeenth day of incubation in eggs from hens fed a diet deficient in vitamin B_{12} (154). Vitamin B_{12} prevented fatty content and weight increase of livers in rats fed a high fat ration (20, 34, 71, 127, 203, 205).

A relationship between vitamin B_{12} and folic acid (19, 60, 68, 69, 70, 147, 148, 167, 183, 187, 193, 210), pantothenic acid (150), vitamin C (69), choline (37, 182, 184, 185, 186, 187, 204, 205), vitamin E (103), thymidine (195), phosphorus (1, 172), intrinsic factor (164), hydrocephalus in mice (151) and enzymatic activity (222) has been reported.

Swine

Since the isolation of vitamin B_{12}, a large amount of work has been done which shows its growth promoting value in swine nutrition (6, 8, 18, 35, 38, 39, 45, 46, 47, 56, 63, 64, 65, 66, 74, 75, 93, 102, 105, 119, 128, 129, 130, 143). Although the amount of work with vitamin B_{12} concentrates and crystalline vitamin B_{12} has been great, relatively little is known about its quantitative requirement by swine.

Anderson and Hogan (7) fed a synthetic ration to two day old pigs. Basing the vitamin B_{12} requirements on one pig and assuming that injection is twice as effective as oral administration, they set the tentative requirement for vitamin B_{12} when administered orally at 0.26 microgram daily per kilogram of body weight or not over 1.5 micrograms per 100 grams of feed. Nesheim et al. (140, 141) have shown that the small pig under 8 weeks of age requires approximately 9 micrograms of vitamin B_{12} per pound of total ration on a dry basis. Neuman et al. (142) estimated the vitamin B_{12} requirement of the baby pig when fed in the form of a concentrate in a soybean protein synthetic milk to be

42 micrograms of vitamin B_{12} per kilogram of dry matter in the ration. Based on a later experiment (145), they stated the requirement to be approximately 50 micrograms per kilogram of diet.

In early work with growing fattening pigs, Catron and Culbertson (47) estimated the vitamin B_{12} requirement to be 10 micrograms per pound of total ration when a standardized vitamin B_{12} concentrate was fed. Lepley (119) using a Merck concentrate containing two milligrams of vitamin B_{12} per pound of concentrate obtained 1.67, 1.78, 1.80 and 1.87 pounds daily gain with growing-fattening pigs fed levels of 0, 5, 10, and 15 micrograms of vitamin B_{12} per pound of an all plant ration. However, the addition of 0, 5, 10 and 20 micrograms of crystalline vitamin B_{12} to a fortified corn-soybean oil meal ration containing 2 per cent dried whey did not give any response in weight gains (120). The dried whey probably supplied sufficient vitamin B_{12} for the pig. Vohs et al. (218) concluded that the practical vitamin B_{12} requirement of growing-fattening pigs from weaning to 200 pounds in concrete drylot does not exceed 5 micrograms per pound when added to a corn-soybean oil meal ration. He fed a chick assayed vitamin B_{12} concentrate at levels of 0, 5, 10 and 15 micrograms per pound of total ration with an additional group of pigs fed crystalline vitamin B_{12} as a reference standard.

Poultry

As with swine, a large amount of experimental work has been done with vitamin B_{12} in poultry feeding; however, the work done to determine the requirement is relatively small. Ott (156), using a purified diet containing 40 to 70 per cent soybean oil meal stated that the optimal growth requirement of the chick appears to be less than 30 micrograms per kilogram of diet. Hogan (101) gave 1.5 micrograms vitamin B_{12} per 100 grams of diet as the requirement for baby chicks. Menge (136) found that the addition of 1-4 per cent glycine to the diet of vitamin B_{12} deficient chicks depressed growth. As little as 3 micrograms of vitamin B_{12} per kilogram of ration overcame this effect.

Peterson et al. (160) showed that levels of 1 to 4 micrograms vitamin B_{12} per hen weekly injected into hens on a deficient diet resulted in maximum hatchability. At the 4 microgram level, there was sufficient carryover to permit excellent chick growth.

In experimental work at Cornell (150), the minimum amount of vitamin B_{12} to promote satisfactory growth in chicks of hens whose eggs gave normal hatchability, although below normal in vitamin B_{12} content, was found to be 1.13 micrograms per pound of ration. The vitamin B_{12} per gram of egg yolk in eggs of hens giving 60 per cent hatchability was 3.1 micrograms per gram of yolk; yolk from hens giving 83 per cent hatchability contained 4.1 micrograms per gram of yolk; eggs of hens from which hatchery chicks were obtained contained 10.5 micrograms per gram of egg yolk. Studies with breeders indicated that the minimum vitamin B_{12} requirement for hatchability is approximately one microgram per pound of ration.

Milligan and Combs (138) found that four micrograms of vitamin B_{12} per kilogram of ration supported good hatchability and viability of progeny. Johnson (107) states that not more than one microgram of vitamin B_{12} per kilogram of diet is necessary for hatchability.

EXPERIMENTAL

Experiment I

Plan

The purpose of this experiment was to determine the nutritional requirement of vitamin B_{12} for the male pig from weaning to 75 pounds.

Six Duroc litters containing four male pigs each were selected at weaning. Great care was taken to select only thrifty pigs which were as uniform in all respects as possible. Litter mates were randomly allotted to the various treatments.

During gestation, the dams of these pigs received an adequate 16 per cent protein ration containing animal protein, minerals including trace minerals and additional vitamins A, D_2, riboflavin, niacin and pantothenic acid. During lactation, an all-plant ration was fed. It was fortified only with minerals including trace minerals and vitamins A and D_2.

After allotment of the pigs to the different treatments they were sprayed with benzene hexachloride and each placed in an individual crate. All pigs received the basal ration for two days. On the third day, a 24 hour urine and feces sample was collected and stored at -23° C. Several collections were made during the 24 hour period and stored in the refrigerator until the total 24 hour sample was obtained. Each sample was covered with toluene to prevent further bacterial action. During the fourth day, all pigs were wormed with the basal ration containing one per cent sodium flouride. On the fifth day, the pigs were weighed and started on the assigned experimental ration.

Each pig was kept in an 18 by 40 inch wood crate with wire floor. Individual self-feeders and watering pans were used. The crates were thoroughly washed 3-4 times daily as an extra precaution in preventing coprophagy. A picture of the type feeding crate used is shown in Figure 1.

The basal ration, consisting of ground yellow corn and blended solvent soybean oil meal plus a complex vitamin mixture and minerals including trace minerals, is shown in Table 1. To this ration, levels of 0, 5, 10 and 20 micrograms of crystalline vitamin B_{12} were added per pound of ration. The treatments were subdivided, one-half of the pigs receiving 40 milligrams of antibiotics per pound of total ration. This was an attempt to control the intestinal flora. The antibiotic mixture consisted of 10 milligrams each of aureomycin hydrochloride, streptomycin sulfate, terramycin hydrochloride and procaine penicillin G.

An individual record of feed consumption and gain in weight was made on each pig at weekly intervals. After reaching 75 pounds of weight, each pig's record was terminated. A 24 hour collection of urine and feces was made as previously described. These samples were collected to be assayed for vitamin B_{12} content. Blood samples were obtained from each pig as it came off experiment to determine hemoglobin, red and white blood cell counts, total nitrogen and urea.

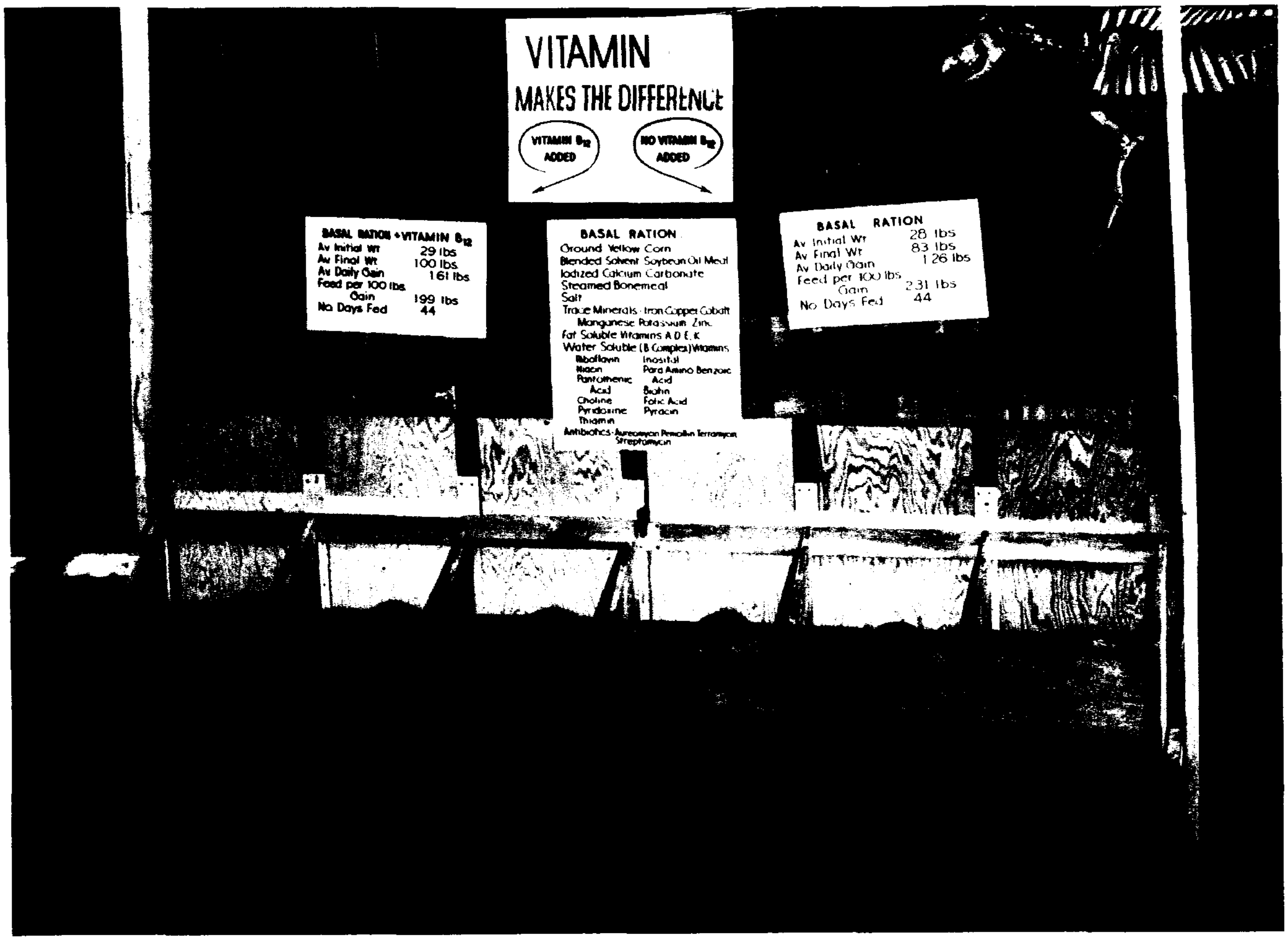

Figure 1. Front view of crates and feeders used in these experiments

Table 1. Composition of the basal ration

	%
Ground yellow corn	71.0
Solvent soybean oil meal (blended)	24.5
Vitamin Premix No. 5[a]	1.0
Special steamed bonemeal	2.2
Iodized calcium carbonate	0.7
Iodized salt	0.5
Trace mineral mixture[b]	0.1

Calculated analysis		Chemical analysis			
		Lot	Protein %	Calcium %	Phosphorus %
Protein, %	18.03				
Fat, %	2.88				
Fiber, %	3.57	1	18.50	1.07	0.63
Calcium, %	.987	2	17.50	0.92	0.59
Phosphorus, %	.643	3	19.25	1.06	0.64
Vit. A, I.U. per lb.	2079.00	4	17.68	1.01	0.62
Vit. D_2, U.S.P. units per lb.	400.00	5	19.46	1.13	0.66
Riboflavin, Mg. per lb.	1.66	6	17.85	0.94	0.58
Niacin, Mg. per lb.	18.58	7	17.85	0.99	0.59
Pantothenic acid, Mg. per lb.	7.95	8	17.90	1.13	0.65
Choline, Mg. per lb.	749.00				

[a]Vitamin Premix No. 5 supplied the following amounts of vitamins per pound of ration:

Alpha-tocopherol acetate	1.5 mg	Para amino benzoic acid	0.5 mg
Biotin	0.2 "	Pyracin	1.0 "
Calcium pantothenate	5.0 "	Pyridoxine	1.5 "
Choline chloride	250.0 "	Riboflavin	1.0 "
Folic acid	0.5 "	Thiamin	1.5 "
Inositol	200.0 "	Vitamin K	0.4 "
Niacin	10.0 "	Vitamin A	300.0 I.U.
		Vitamin D_2	400.0 U.S.P. units

[b]Contributed the following ppm to the ration: Fe-70, Co-1.6, Cu-4.8, Mn-59, Zn-4.4 and K-7.6.

Results and Discussion

Table 2 gives the weekly weights of the pigs. Table 3 gives a summary of the growth results. There was a slight increase in average daily gain over the basal as 5, 10 and 20 micrograms of vitamin B_{12} were added per pound of ration. Only the 20 microgram level gave a significantly ($p = .05$) faster gain over the basal. The pigs receiving the basal ration including antibiotics gained significantly ($p = .01$) less than those receiving the basal ration alone. It appears that the antibiotics suppressed the intestinal microflora including those which synthesize vitamin B_{12} and also those that might compete with the pig for vitamin B_{12}. The pigs fed the basal ration grew poorly because no vitamin B_{12} was supplied in the ration and apparently vitamin B_{12} synthesis in the intestinal tract was inhibited by the antibiotics, at least in the part where absorption and utilization could take place.

The average daily gain of all the pigs receiving 40 milligrams of antibiotics per pound of ration was 1.43 pounds, which is significantly greater ($p = .01$) than 1.24 pounds for those receiving no antibiotics. In this case it is suggested that the antibiotics suppressed the intestinal flora which compete with the pig for vitamin B_{12}, thus making more of the vitamin B_{12}, which was added in the ration, available for the pig.

The addition of vitamin B_{12} at all levels with antibiotics produced highly significantly($p = .01$) faster gains than the basal with antibiotics

Table 2. Weekly weights of pigs

Pig number	Starting Date	Starting Weight	May 24	May 31	June 7	June 14	June 21	June 28	July 5	July 12	July 19	July 26	Aug. 2	Aug. 10
						Lot I. Basal								
34	5/15	32.5	47.0	55.0	67.0	74.0								
						75.0	June 17							
43	5/18	37.0	43.0	50.0	61.0	69.0								
						75.0	June 19							
125	5/22	30.5	33.0	37.5	46.0	55.5	67.0	73.0						
								75.0	July 2					
						Lot II. Basal + 5 micrograms vitamin B_{12} per lb. ration								
35	5/15	33.5	46.5	54.0	63.5	72.5								
						75.0	June 17							
44	5/18	35.0	42.5	51.0	59.0	67.0								
						75.0	June 20							
126	5/22	31.0	35.0	42.5	50.0	58.5	67.0	75.0						
						Lot III. Basal + 10 micrograms vitamin B_{12} per lb. ration								
30	5/15	32.5	43.5	52.5	62.0	70.5								
						75.0	June 17							
45	5/18	38.0	43.5	56.0	66.0	72.0								
						75.0	June 17							
127	5/22	32.0	35.5	45.0	53.5	62.5	71.0							
							75.0	June 26						

Table 2. (continued)

Pig number	Starting Date	Starting Weight	May 24	May 31	June 7	June 14	June 21	June 28	July 5	July 12	July 19	July 26	Aug. 2	Aug. 10
						Lot IV. Basal - 20 micrograms vitamin B_{12} per lb. ration								
31	5/15	30.5	43.0	53.5	64.5	74.0								
						75.0	June 16							
40	5/18	37.5	43.5	53.0	61.5	74.0								
						75.0	June 17							
122	5/24	28.5	28.5	37.0	48.0	56.5	64.5	72.0						
								75.0	July 1					
						Lot V. Basal + 40 mg. antibiotics								
64	5/19	25.5	29.0	31.5	39.0	50.0	56.5	65.5	75.0					
95	5/21	33.0	37.0	45.0	52.0	62.5	66.5	72.0						
								75.0	July 2					
634	6/4	29.0	-	-	33.0	38.5	45.5	46.0	52.5	60.5	64.0	67.5	70.0	75.0
						Lot VI. Basal - 5 micrograms vitamin B_{12} per lb. ration + 40 mg. antibiotics								
62	5/19	31.0	39.5	48.5	59.5	71.5								
						75.0	June 17							
90	5/21	33.0	37.5	48.5	59.0	70.0								
						75.0	June 17							
631	6/4	27.0	-	-	30.5	44.0	53.5	62.5	75.0					

Table 2. (concluded)

Pig number	Starting Date	Starting Weight	May 24	May 31	June 7	June 14	June 21	June 28	July 5	July 12	July 19	July 26	Aug. 2	Aug. 10
			Lot VII. Basal + 10 micrograms vitamin B_{12} per lb. ration + 40 mg. antibiotics											
61	5/19	24.5	30.0	41.5	52.5	68.0								
						75.0	June 19							
96	5/21	34.0	39.0	49.5	62.0	73.0								
						75.0	June 16							
635	6/4	24.5	-	-	27.5	39.0	50.0	59.0	73.0					
									75.0	July 7				
			Lot VIII. Basal - 20 micrograms vitamin B_{12} per lb. ration - 40 mg. antibiotics											
60	5/19	30.5	37.0	43.5	59.0	69.0								
						75.0	June 19							
92	5/21	30.5	35.0	46.5	57.5	70.0								
						75.0	June 19							
633	6/4	24.5	-	-	29.5	42.0	52.5	59.5	75.0					

Table 3. Average daily gains in pounds of individual pigs to 75 pound live weight

Level of vitamin B_{12} (mcg. per lb. ration)		0	5	10	20	Average of all levels
No antibiotics	Litter 30	1.30	1.26	1.29	1.38	
	" 40	1.19	1.21	1.23	1.27	
	" 120	1.08	1.19	1.23	1.22	
	Average	1.19	1.22	1.25	1.29*	1.24
Antibiotics	Litter 60	1.05	1.52	1.63	1.44	
	" 90	1.00	1.56	1.57	1.53	
	" 630	0.69	1.55	1.53	1.63	
	Average	0.91	1.54**	1.58**	1.53**	1.43**

*Significant at P = 0.05.

**Significant at P = 0.01.

alone. However, there were no differences in the growth responses between the 5, 10 and 20 microgram levels. Vitamin B_{12}, in the presence of antibiotics, gave a significantly higher (p = .01) rate of gain than when vitamin B_{12} was fed without antibiotics.

The addition of vitamin B_{12} alone increased feed efficiency but not significantly. Antibiotics with vitamin B_{12} gave a significant decrease (p=.01) of 42 pounds of feed per 100 pounds gain. Table 4 shows the average daily feed consumption per pig and feed required per 100 pound of gain.

A blood sample was obtained from each pig as it reached 75 pounds. There were no significant differences in hemoglobin, red and white blood cell counts or total nitrogen on any of the treatments. Antibiotics added to the basal ration increased blood urea; however, antibiotics

Table 4. Average daily feed consumed per pig and feed required per 100 pounds gain

Levels of vitamin B_{12} (mcg. per lb. ration)	0	5	10	20	Average of all levels
Average daily feed (lb.)					
No antibiotics	3.20	2.87	3.10	3.00	3.04
Antibiotics	2.10	3.01	3.02	2.94	2.77
Average	2.65	2.94	3.06	2.97	
Feed per 100 lbs. gain (lb.)					
No antibiotics	265	236	248	233	246
Antibiotics	235	195	192	193	204**
Average	250	216	220	213	

**Significant at P = 0.01.

plus vitamin B_{12} lowered the blood urea. Apparently vitamin B_{12} enhanced the utilization of nitrogen. A summary of these results is given in Table 5.

Microbiological assay of the urine and feces was carried out using the basal medium given by Peeler et al. (159) and the procedure as worked out by Friedland (84). Lactobacillus leichmannii 313 was used as the assay organism. While there is much to be desired in the improvement of the microbiological assay of vitamin B_{12} in biological substances, it is felt that the method used is as good or better than any other published procedure to date.

Microbiological assay of the urine showed very small amounts of vitamin B_{12}; however, the trend is upward as the vitamin B_{12} is increased in the feed. The results are in fair agreement with those obtained by Chas. Pfizer and Co., Inc., on the same sample collections. Table 6 gives a summary of these results.

Table 5. Summary of blood data

Pig number	Hemoglobin, grams per 100 ml. blood	Red blood cell count x 1000	White blood cell count	Total nitrogen, grams per 100 ml.	Urea, milligrams per cent
	Lot I. Basal				
34	16.0	5,855	14,800	3.07	15.8
43	11.8	8,160	28,040	2.81	12.1
125	13.0	5,800	17,480	2.62	15.3
Average	13.6	6,605	20,107	2.83	14.4
	Lot II. Basal + 5 micrograms vitamin B_{12}				
35	12.8	6,180	21,880	3.38	-
44	13.0	2,225	9,750	3.11	15.9
126	11.9	5,540	19,320	3.16	14.0
Average	12.6	4,648	16,983	3.22	15.0
	Lot III. Basal + 10 micrograms vitamin B_{12}				
30	14.8	5,910	18,920	3.26	-
45	16.1	5,600	17,800	3.34	13.2
127	12.7	5,170	18,800	3.37	18.0
Average	14.5	5,560	18,507	3.32	15.6
	Lot IV. Basal + 20 micrograms vitamin B_{12}				
31	12.3	5,333	13,840	2.61	-
40	14.4	6,270	24,920	2.77	15.4
122	12.0	4,270	20,720	2.87	14.1
Average	12.9	5,291	19,827	2.75	14.8
	Lot V. Basal + 40 milligrams antibiotics				
64	12.9	7,353	16,960	3.17	15.0
95	13.1	4,100	14,320	2.80	18.2
634	12.0	5,920	8,520	2.91	28.8
Average	12.7	5,791	13,267	2.99	20.7

Table 5. (continued)

Pig number	Hemoglobin, grams per 100 m. blood	Red blood cell count x 1000	White blood cell count	Total nitrogen, grams per 100 ml.	Urea, milligrams per cent
	Lot VI. Basal + 5 micrograms vitamin B_{12} + 40 milligrams antibiotics				
62	16.1	5,940	18,320	3.21	8.6
90	16.0	8,570	15,320	3.03	-
631	14.9	7,850	18,640	2.96	9.6
Average	15.7	7,453	17,427	3.07	9.1
	Lot VII. Basal + 10 micrograms vitamin B_{12} + 40 milligrams antibiotics				
61	10.6	5,540	17,960	3.36	12.5
96	11.4	7,780	17,640	2.94	-
635	15.0	7,540	20,280	3.45	9.7
Average	12.3	6,953	18,627	3.25	11.1
	Lot VIII. Basal + 20 micrograms vitamin B_{12} + 40 milligrams antibiotics				
60	12.8	8,340	15,000	3.11	6.9
92	10.4	5,920	16,440	3.00	-
633	12.5	6,850	14,680	3.10	7.8
Average	11.9	7,037	15,373	3.07	7.4

Table 6. Summary of vitamin B_{12} assay of urine

Micrograms vitamin B_{12} per pound of ration	Average milliliters urine in 24 hours	Milli-micrograms[a] of B_{12} per milliliter	Milli-micrograms[b] of B_{12} per milliliter	Milli-micrograms of B_{12} in 24 hours
No antibiotics				
0	737	1.24	1.50	914
5	600	1.59	1.80	954
10	703	2.76	2.40	1940
20	750	2.79	2.30	2092
Antibiotics (40 mg. per pound of ration)				
0	237	0.62	0.83	147
5	657	1.17	1.50	769
10	783	1.78	1.50	1394
20	613	3.43	2.00	2103

[a]Iowa State College, Ames, Iowa.

[b]Chas. Pfizer and Co., Inc., Brooklyn, New York.

As in the case of the urine, an increase of vitamin B_{12} was found in the feces as the amount fed was increased except on the 10-microgram level with antibiotics. No explanation can be given for the seeming discrepancy in this value. A summary of the fecal assays is presented in Table 7. The results are in very good agreement with those obtained by Chas. Pfizer and Co., Inc. on the same samples. In all cases, the results of fecal vitamin B_{12} assays show a much greater amount of vitamin B_{12} than can be accounted for in the feed. Considering these values as correct, it is evident that synthesis of vitamin B_{12} is taking place in the digestive tract of the pig.

Table 7. Summary of vitamin B_{12} assay of feces

Micrograms vitamin B_{12} per pound of ration	Average grams of feces in 24 hours	Micrograms[a] of B_{12} per gram	Micrograms[b] of B_{12} per gram	Micrograms of B_{12} in 24 hours
No antibiotics				
0	229	0.85	0.86	195
5	206	0.95	0.99	196
10	486	1.00	1.10	486
20	411	1.35	1.26	555
Antibiotics (40 mg. per pound of ration)				
0	130	0.80	0.80	104
5	388	1.15	1.24	446
10	492	0.70	0.52	344
20	263	2.50	2.07	658

[a]Iowa State College, Ames, Iowa.

[b]Chas. Pfizer and Co., Inc., Brooklyn, New York.

The feeding of antibiotics apparently did not interfere with the assay. This is probably because of the high dilution factor in assaying. Alkali destruction by steaming the samples in 0.2N potassium hydroxide and paper strip chromatography showed that 97 to 98 per cent of the growth promoting activity for Lactobacillus leichmannii 313 was due to vitamin B_{12} and not to thymidine or other alkali-stable growth promoting factors.

One pig from each treatment was sacrificed at the end of the experiment to obtain the liver. The results of the liver assays are presented in Table 8. Based upon these results, it is apparent that the feeding of 10 micrograms of vitamin B_{12} per pound of ration allows maximum storage in the liver.

Table 8. Summary of vitamin B_{12} assay of liver

Micrograms vitamin B_{12} per pound of ration	Total grams	Micrograms[a] of B_{12} per gram	Total micrograms of B_{12}
No antibiotics			
0	794	0.057	45
5	681	0.082	56
10	735	0.152	112
20	796	0.130	103
Antibiotics (40 mg. per pound of ration)			
0	732	0.056	41
5	687	0.083	57
10	686	0.144	99
20	667	0.133	89

[a] Chas. Pfizer and Co., Inc., Brooklyn, New York.

Pigs receiving the basal ration and antibiotics without additional vitamin B_{12} exhibited vitamin B_{12} deficiency as evidenced by an extremely rough hair coat, a dermatitis which could not be alleviated by spraying with benzene hexachloride and a brownish exudate around the eyes. They were extremely nervous and irritable. These pigs had a tendency to be constipated as evidenced by lumpy, greenish-grey feces. The feces of all other pigs appeared normal at all times. None of the pigs ever scoured. Pigs receiving vitamin B_{12} did not show any deficiency symptoms.

Pictures of litter mates showing typical response to crystalline vitamin B_{12} in the presence of antibiotics are shown in Figure 2. All pigs received 40 milligrams of antibiotics per pound of ration. Pigs 5, 6, 7 and 8 received 0, 5, 10 and 20- micrograms respectively of crystalline

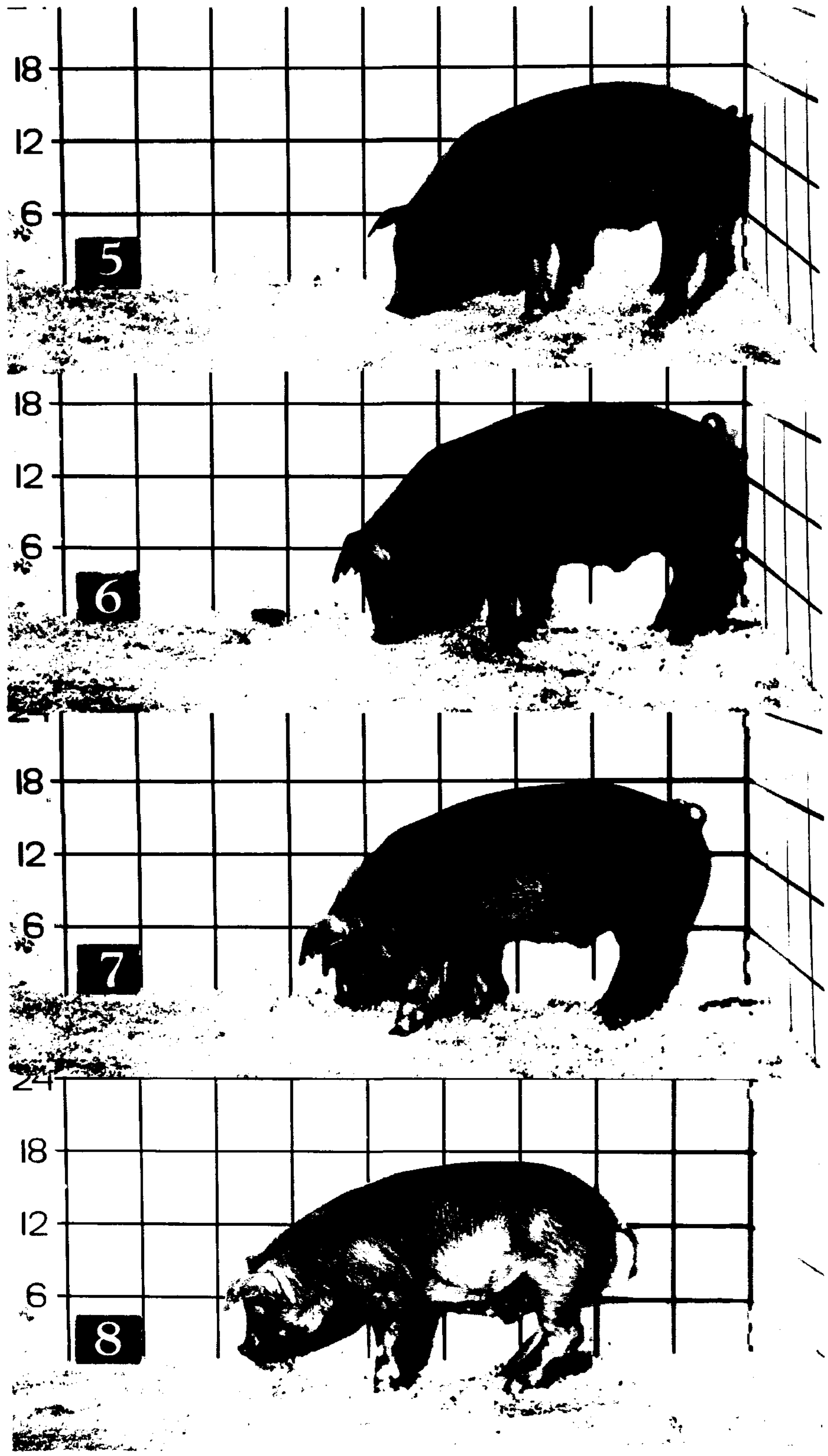

Figure 2. Litter 60 showing typical response to crystalline vitamin B_{12} fed levels of 0, 5, 10 and 20 micrograms per pound of ration in the presence of antibiotics

vitamin B_{12} per pound of ration. These pictures were taken after the pigs had been on experiment 24 days. The gains of 5, 6, 7 and 8 were 21, 37, 34 and 35.5 pounds, respectively, for this period.

One pig which showed vitamin B_{12} deficiency and exhibited growth stasis was injected intraperitoneally with 10 micrograms of crystalline vitamin B_{12} when it weighed 75 pounds. It was kept on the same ration for one week and then 10 micrograms of vitamin B_{12} were added to each pound of ration for three more weeks. During the four weeks period, the pig gained 35 pounds on 75 pounds of feed. This was an average daily gain of 1.25 pounds as compared with the previous average daily gain of 0.69 pound before it received vitamin B_{12}.

Summary

Six sets of four male litter mate pigs, three per treatment, were individually fed and watered, ad libitum, from weaning to 75 pounds in wire floored crates. The pigs were fed a fortified corn-soybean oil meal ration to which was added 0, 5, 10 and 20 micrograms of crystalline vitamin B_{12} per pound of ration. The ration treatments were subdivided with one-half of the pigs receiving 40 milligrams of antibiotics per pound of ration.

Vitamin B_{12} alone produced only slight increases in daily gain but in the presence of antibiotics the increases were highly significant. There were no significant differences in response between the added levels of vitamin B_{12} as judged by gains of the pigs. The addition of an antibiotic mixture to the ration increased average daily gains and feed efficiency significantly. There were no differences in hemoglobin, red and white blood cell counts or total nitrogen in the blood. Antibiotics alone increased blood urea; however, when vitamin B_{12} was added, the blood urea was lowered. There was an increase of vitamin B_{12} in the urine as the amount in the ration increased. This was also true with the feces, except for the 10-microgram level with antibiotics. Likewise, there was an increase in the vitamin B_{12} content of the liver as the levels of vitamin B_{12} were increased in the rations.

The weanling pig needs vitamin B_{12} for optimum growth and feed efficiency. With intestinal microflora control by the use of a combination of antibiotics, the vitamin B_{12} requirement of the weanling pig is 5 micrograms or less per pound of ration when added to a corn-soybean meal diet.

Pilot Experiment I.

Plan

The purpose of this experiment was to study the requirement, storage and length of time of effective carryover of vitamin B_{12} in weanling pigs from dams which had received vitamin B_{12}. The dams of these pigs were fed an adequate ration including meat and bone scraps from weaning till breeding. They were confined in concrete drylots during gestation and lactation. Their ration consisted of ground yellow corn, soybean oil meal, dried whey, vitamins A, D_2, riboflavin, niacin and pantothenic acid, minerals including trace minerals and five micrograms of crystalline vitamin B_{12} per pound of ration. The pigs had access to the ration of their dams at all times.

After allotment, the pigs were fed the same basal ration and managed the same as explained in Experiment I. Lot I received the basal ration; Lot II, basal plus 10 micrograms of vitamin B_{12}; Lot III, basal plus 10 micrograms vitamin B_{12} and 40 milligrams antibiotics; Lot IV, basal plus 40 milligrams of antibiotics. The antibiotic mixture consisted of 10 milligrams each of aureomycin hydrochloride, terramycin hydrochloride, procaine penicillin G and streptomycin sulfate.

Results and discussion

Table 9 gives the weekly weights of the pigs. Average daily gains and feed consumption are presented in Table 10. Based upon the appearance of the pigs and rates of gain, the pigs receiving the basal ration plus

Table 9. Weekly weights of pigs

Pig number	8-30	9-6	9-13	9-20	9-27	10-4	10-13	10-19
			Lot I. Basal ration					
1240B	33	41	53	61	68	80		
1244S	32	44	53	61	66	78		
1256S	26	31	38	45	52	64		
Average	30.3	38.7	48.0	55.7	62.0	74.0		
			Lot II. Basal + 10 micrograms vitamin B_{12}					
1242B	31	44	45	51	56	68		
1245S	34	45	55	60	67	78		
Average	32.5	44.5	50.0	55.5	61.5	73.0		
			Lot III. Basal + 10 micrograms vitamin B_{12} + 40 milligrams antibiotics					
1241B	24	37	46	58	67	77	92	100
1246S	34	49	58	69	78	90	106	111
1250B	29	42	50	60	75	86	101	105
Average	29.0	42.7	51.0	62.3	73.3	84.3	99.7	105.3
			Lot IV. Basal + 40 milligrams antibiotics					
1243B	31	43	50	64	75	78	88	93
1247S	25	34	40	53	60	70	76	80
1251B	27	35	42	53	65	72	85	89
Average	27.7	37.3	44.0	56.7	66.7	73.3	83.0	87.3

Table 10. Average daily gain and feed consumption

Lot	Addition to basal ration (per pound)	Average daily gain (lbs.)	Average daily feed (lbs.)	Feed per 100 lbs. gain (pounds)
		35 days on experiment		
I	None	1.25	2.77	222
II	10 micrograms of vitamin B_{12}	1.16	2.86	247
III	10 micrograms of vitamin B_{12} plus 40 milligrams of antibiotics	1.58	3.15	200
IV	40 milligrams of antibiotics	1.30	2.89	221
		50 days on experiment		
III	10 micrograms of vitamin B_{12} plus 40 milligrams of antibiotics	1.53	3.63	238
IV	40 milligrams of antibiotics	1.19	3.17	265

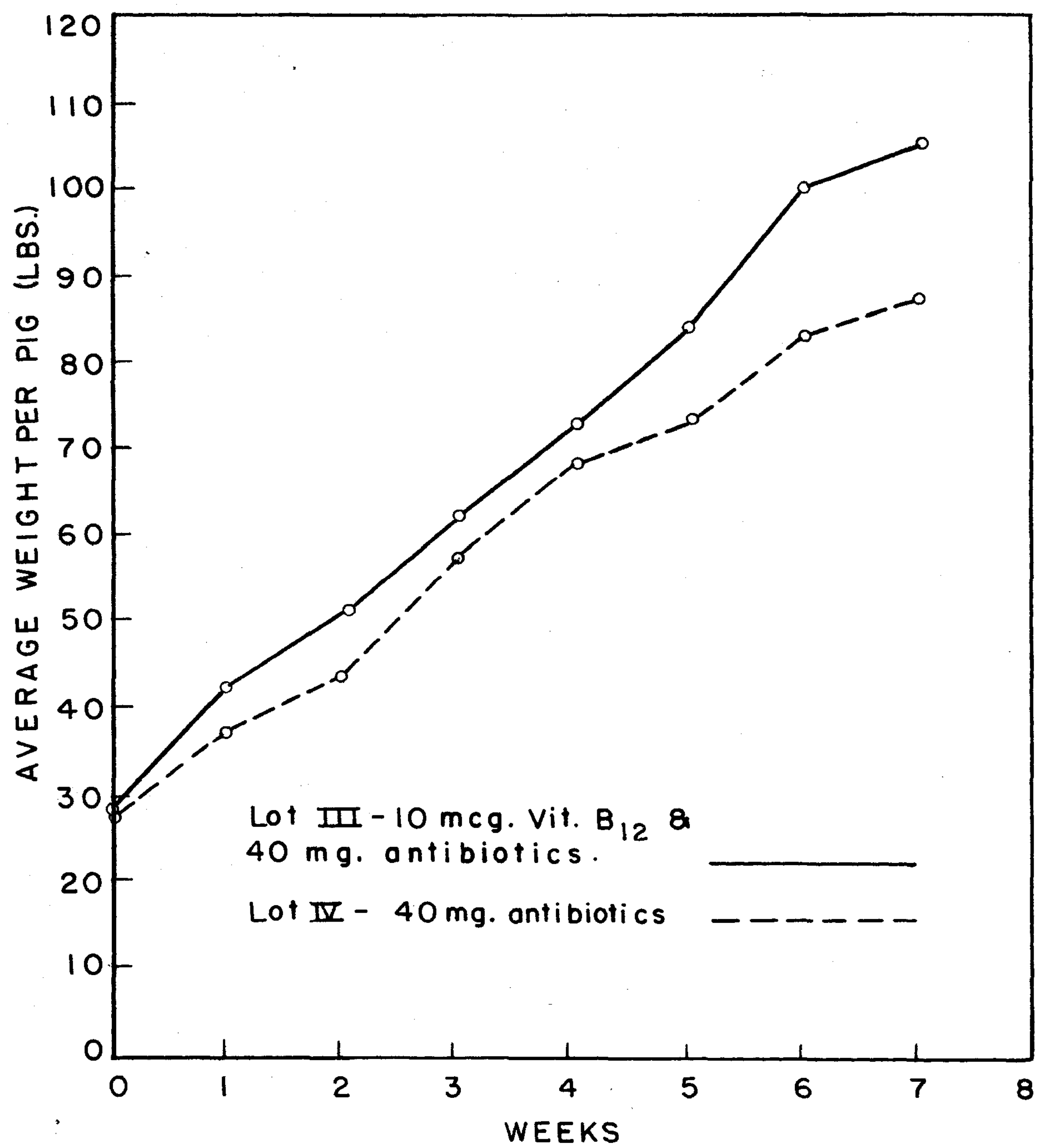

Fig. 3. Growth Curves of Lot Mean Weights.

Table 11. Summary of blood data

Pig number	Hemoglobin grams per 100 ml.		Red blood cell count x 1,000		White blood cell count		Total nitrogen, grams per 100 milliliters		Urea, milligrams per cent	
	Start exp.	End exp.	Start exp.	End exp.	Start exp.	End exp.	Start exp.	End exp.	Start exp.	End exp.
			Lot I. Basal ration							
1240B	12.2	13.0	7730	3010	22840	16000	3.18	2.48	8.0	5.0
1244S	10.9	11.6	6250	7210	31400	20340	3.04	3.24	13.0	20.0
1256S	7.1	12.0	4840	7330	21360	21440	2.42	-	17.0	-
Average	10.1	12.2	6273	5850	25200	19260	2.88	2.86	12.7	12.5
			Lot II. Basal + 10 micrograms vitamin B_{12}							
1242B	11.0	13.0	7160	6140	21600	23600	2.83	3.18	25.0	12.0
1245S	12.0	13.6	8720	7710	10720	23400	2.76	3.49	27.0	17.0
Average	11.5	13.3	7940	6925	16160	23500	2.80	3.33	26.0	14.5
			Lot III. Basal + 10 micrograms vitamin B_{12} + 40 milligrams antibiotics							
1241B	11.1	12.4	-	6030	-	26040	2.73	-	23.0	25.0
1246S	12.2	13.2	7480	6520	16880	19500	2.77	3.38	11.0	8.0
1250B	8.7	12.1	5820	6330	19200	16880	2.70	2.70	11.0	17.0
Average	10.7	12.6	6650	6293	18040	20806	2.73	3.04	15.0	16.6
			Lot IV. Basal + 40 milligrams antibiotics							
1243B	11.5	13.3	7580	7480	14480	24880	3.10	2.66	23.0	15.0
1247S	11.8	10.8	7810	8350	28400	21620	2.75	3.05	15.0	18.0
1251B	4.5	12.8	4720	7690	28600	19760	1.78	3.36	17.0	24.0
Average	9.3	12.3	6703	7840	23827	22086	2.54	3.02	18.3	19.0

40 milligrams of antibiotics began to show signs of vitamin B_{12} depletion after being on this ration about five weeks. Growth curves for Lots III and IV in Figure 3 show the difference in rate of gain. Table 11 gives a summary of blood data at the beginning and end of the experiment. There were no significant differences at the end of the experiment in the blood data.

Summary

Weanling pigs, from dams receiving vitamin B_{12}, were fed a fortified corn-soybean oil meal ration. Pigs fed the basal ration plus antibiotics began to show signs of vitamin B_{12} depletion after about five weeks as evidenced by a decreased rate of growth. Blood studies at the end of the experiment did not show any significant differences in hemoglobin, red and white blood cells, total nitrogen or urea.

Pilot Experiment II.

Plan

In view of the results obtained with the pigs on the basal ration plus antibiotics in Experiment I, it seemed advisable to obtain information on the feeding of higher levels of antibiotics before the second experiment was started. Suitable pigs were not available for an experiment; however, four pigs were selected from those left after their litter mates had been alloted to other experiments. Four rations were prepared. The basal ration was the same as the one used in Experiment I without antibiotics. To the other three rations, antibiotics were added at the rate of 40, 80 and 200 milligrams of antibiotics per pound of ration. The antibiotic mixture consisted of aureomycin hydrochloride, terramycin hydrochloride, streptomycin sulfate and procaine penicillin G at the rate of 10, 20 and 50 milligrams each in the respective rations. The pigs were fed and watered *ad libitum* in wire floored crates.

Results and discussion

The pig receiving the basal ration without antibiotics developed bloody scours. It was removed as a precaution against further spread of this disorder. Observations made on the pigs did not show any marked difference in appetite, thriftiness, rate of gain, bloom and general condition. The Iowa Veterinary Diagnostic Laboratory findings are given in the two following reports.

Report No. 1--January 11, 1951. "Examination of the three pigs submitted January 8 from Experiment 505B has revealed the following information:

"Pig No. 3340B (fed 200 milligrams antibiotics per pound of ration) showed the presence of no gross lesions. No cecal protozoa were demonstrated from this specimen. Bacterial cultures prepared on blood agar cultivated in aerobic conditions have revealed the presence of only a moderate growth of _Escherichia spp._ from the large intestine. No _Proteus spp._ or _Pseudomonas spp._ were recovered from these cultures. The food material in the digestive tract appeared to be in better state of preservation than that commonly seen in the lower digestive tract of pigs. Whether this is due to the preservative action of the antibiotics administered this pig is difficult to evaluate.

"Pig No. 3375B (fed 80 milligrams antibiotics per pound of ration) failed to reveal the presence of any gross lesions. The cecal content of this pig contained large numbers of _Trichomonas suis_. An abundant growth of _Escherichia spp._ was obtained from the large intestine and a moderate growth from the small intestine. As in the previous pig, the intestinal content of this specimen was also found to be in a better state of preservation than expected in the digestive tracts of normal pigs.

"Pig no. 359B (fed 40 milligrams antibiotics per pound of ration) failed to reveal the presence of any gross lesions except a very few areas of bronchial pneumonia involving a few scattered areas of the lung. No cecal protozoa were demonstrated. An abundance of _Escherichia spp._

was obtained from the large and small intestines. The intestinal content of this pig was in better than average state of preservation.

"We are preparing tissue sections from the kidney, liver and spleen of these three pigs and will notify you of the histological findings as these species are available."

Report No. 2--January 31, 1951. "Examination of the three pigs submitted January 8 from Experiment no. 505B failed to reveal any gross lesions as we reported to you previously. We have prepared tissue sections from the liver, kidney and spleen from each of these specimens and have examined these for the presence of cellular changes.

"Pig no. 3340B (fed 200 milligrams antibiotics per pound of ration) exhibited fatty swelling of the renal tubules. The spleen was normal. Cloudy swelling of the liver cord cells was present.

"Pig no. 3375B (fed 80 milligrams of antibiotics per pound of ration) was found to be affected with cloudy swelling of the renal tubules. The spleen appeared normal. The liver showed cloudy swelling of the liver cord cells.

"Pig no. 359B (fed 40 milligrams of antibiotics per pound of ration) presented a normal appearing spleen. The liver showed cloudy swelling of the liver cord cells and in addition it showed a few foci of neutrophillic infiltration indicating the initial phase of necrosis. The kidney exhibited a cloudy swelling of the renal tubules and showed the presence of a slight fibrous proliferation indicating the initial stages of a slight fibrosis.

"These findings indicate that there have been changes instigated in these tissues. The cloudy swelling of the liver cord cells was especially prominent in the central portion of the liver nodules. We hope this information will be of value."

Signed: W.P. Switzer, D.V.M.

Summary

Three weanling pigs were fed a mixture of antibiotics at the rate of 40, 80 and 200 milligrams respectively per pound of ration. Antibiotics produced a marked change in the bacterial flora of the digestive tract. Histological studies indicated that changes were instigated in liver and kidney tissues. While these findings are on a small number of animals, they certainly indicate a fertile field of research to evaluate not only the usefulness of antibiotics in feeding swine but also the effect which they may have on tissues and organs by continuous use.

Experiment II.

Plan

The results of Experiment I showed that 5 micrograms of crystalline vitamin B_{12} added per pound of a fortified corn-soybean oil meal ration gave just as good results with growing-fattening pigs as did greater amounts of vitamin B_{12}. The purpose of this experiment was to obtain further information on the exact nutritional requirement of the weanling pig for vitamin B_{12}.

The feeding and management of the dams and pigs to weaning was the same in this experiment as that described in Experiment I except for a few minor changes which will be pointed out. Considerable difficulty was experienced in raising these pigs during the nursing period due to extremely bad weather and labor conditions. It should also be remembered that the ration being used was formulated to produce pigs as depleted of vitamin B_{12} as practically feasible. Consequently, rigid selection of animals could not be made. Five purebred Duroc and one Duroc-Landrace litters, containing four pigs each of the same sex, were used. Three litters were male and three female.

The 24 hour urine and fecal samples were collected as previously described to determine the amount voided in 24 hours. The fecal sample for assay was collected by holding the pig by the tail and catching the sample in a clean dixie cup as the pig defecated. The urine collection pans were cleaned and the urine collected in another clean dixie cup.

Toluene was immediately applied and the samples deposited in the freezer at 10 degrees Fahrenheit until they were assayed.

The basal ration for this experiment is shown in Table 12. This ration is practically the same as the one used in Experiment I except that 33 milligrams of ascorbic acid and 80 milligrams of a combination of antibiotics were added per pound of ration. To this ration, levels of 0, 2, 4 and 6 micrograms of crystalline vitamin B_{12} were added per pound of ration. Litter mates were allotted at random to the various levels of vitamin B_{12}.

Results and discussion

Table 13 gives the weekly weights of the pigs. Figures 4 through 9 show pictures of the pigs by litters and the length of time on experiment at the time each picture was taken. Pigs 1, 2, 3 and 4 received 0, 2, 4 and 6 micrograms respectively of vitamin B_{12} per pound of ration. The average individual daily gains to 75 and 100 pounds are given in Table 14. An analysis of variance of the average daily gains revealed that the differences among rations as measured by the linear regression on level of vitamin B_{12} were significant at the 1 per cent level of probability.

The average daily gain of 1.739 pounds for the pig in litter 710 receiving 6 micrograms vitamin B_{12} per pound of ration is unusually high. If this value had been consistent with the other daily gains of pigs on 6 micrograms of vitamin B_{12}, it is interesting to note that the average

Table 12. Composition of the basal ration

	%
Ground yellow corn	69.3
Solvent soybean oil meal (blended)	25.2
Vitamin Premix No. 9[a]	1.0
Antibiotic Premix No. 2[b]	1.0
Special steamed bonemeal	2.4
Iodized calcium carbonate	0.5
Iodized salt	0.5
Trace Mineral Mixture[c]	0.1

Calculated analysis		Chemical analysis			
		Lot	Protein %	Calcium %	Phosphorus %
Protein %	18.02				
Fat %	2.77				
Fiber %	3.02	1	19.32	1.02	0.64
Calcium %	0.928	2	18.48	0.41	0.35
Phosphorus %	0.573	3	18.20	0.90	0.60
Vit. A, I.U. per lb.	2079.0	4	18.44	0.96	0.59
Vit. D_2 U.S.P. units per lb.	400.0				
Riboflavin, mg. per lb.	1.67				
Niacin, mg. per lb.	19.70				
Pantothenic acid, mg. per lb.	8.36				
Choline, mg. per lb.	742				

[a]Vitamin Premix No. 9 supplied the following amounts of vitamins per pound of ration:

Alpha-tocopherol Acetate	1.5	mg.	Para amino benzoic acid	0.5	mg.
Ascorbic acid	33		Pyracin	1.0	"
Biotin	0.2	"	Pyridoxine	1.5	"
Calcium pantothenate	5.0	"	Riboflavin	1.0	"
Choline chloride	250.0	"	Thiamin	1.5	"
Folic acid	0.5	"	Vitamin K	0.4	"
Inositol	200.0	"	Vitamin A	300.0	I.U.
Niacin	10.0	"	Vitamin D_2	400.0	U.S.P. units

[b]Antibiotic Premix #2 supplied 20 milligrams each of Aureomycin hydrochloride, Terramycin hydrochloride, Streptomycin sulfate and Procaine penicillin G per pound of ration.

[c]Contributed the following ppm to the ration: Fe-70, Co-1.6, Cu-4.8, Mn-59, Zn-4.4 and K-7.6.

Table 13. Weekly weights of the pigs

Pig number	Jan. 3	Jan. 10	Jan. 17	Jan. 24	Jan. 31	Feb. 7	Feb. 14	Feb. 21	Feb. 28	Mar. 7	Mar. 14	Mar. 21	Mar. 28	Apr. 4	Apr. 11	Apr. 18	Apr. 25	May 2
			Lot I. Basal ration															
774S	18	23	30	38	47	54	59	67	75	78	85	94	100					
734S	12	16	18	24	31	36	37	46	52	52	55	56	59	62	66	72	75*	81
																		92 May 7
953B	-	-	27	38	48	57	61	73	80	85	90	95	100					
								75 Feb. 23										
710B	-	-	36	48	55	62	67	75	80	83	85	88	92	100				
901B	-	-	25	38	40	48	55	62	69	72	77	84	84	90	89**	96	106	
									75 Mar. 10								109 Apr. 28	
965S	-	-	-	20	28	33	39	51	57	62	67	73	78	79	80**	88	97	
												75 Mar. 24					107 Apr. 28	
			Lot II. Basal + 2 micrograms vitamin B_{12} per lb. ration															
778S	14	19	25	31	40	49	56	67	75	86	93							
											100 Mar. 19							
737S	12	17	21	29	38	46	53	64	74	83	90	97						
									75 Mar. 1		100 Mar. 23							
950B	-	-	32	45	57	68	75	87	95									
									100 Mar. 3									
712B	-	-	28	38	48	58	64	69	77	82	85	87	94	99				
								75 Feb. 26						100 Apr. 5				
943B	-	-	24	34	40	43	52	62	69	75	85	90						
												100 Mar. 27						
964S	-	-	-	30	40	47	55	68	77	90	99							
								75 Feb. 26			100 Mar. 15							

*6 micrograms vitamin B_{12} added per pound ration.

**15 micrograms vitamin B_{12} injected and 6 micrograms vitamin B_{12} added per pound ration.

Table 13. (continued)

Pig number	Jan. 3	Jan. 10	Jan. 17	Jan. 24	Jan. 31	Feb. 7	Feb. 14	Feb. 21	Feb. 28	Mar. 7	Mar. 14	Mar. 21	Mar. 28	Apr. 4	Apr. 11	Apr. 18	Apr. 25	May 2
			Lot III. Basal + 4 micrograms vitamin B_{12} per lb. ration															
776S	19	28	33	47	56	66	75	87	96									
									100	Mar. 2								
735S	16	23	28	36	48	58	66	78	90									
							75	Feb.19	100	Mar. 5								
951B	-	-	33	45	55	67	75	87	95									
									100	Mar. 3								
711B	-	-	35	48	56	62	70	82	94									
							75	Feb.16	100	Mar. 4								
900B	-	-	27	42	46	56	67	77	87	96								
							75	Feb. 19		100	Mar. 10							
966S	-	-	-	23	27	34	41	56	63	73	79	86	94					
										75	Mar. 9		100	Apr. 1				
			Lot IV. Basal + 6 micrograms vitamin B_{12} per lb. ration															
779S	14	21	28	35	45	54	60	72	82	89	98							
								75	Feb. 24		100	Mar. 15						
736S	17	23	29	41	52	60	69	84	95									
							75	Feb.16	100	Mar. 3								
952B	-	-	29	42	53	63	72	81	91	97								
							75	Feb.16		100	Mar. 3							
713B	-	-	35	48	61	71	80	91	100									
						75	Feb. 9											
963S	-	-	-	29	43	48	56	68	75	87	91							
											100	Mar. 19						

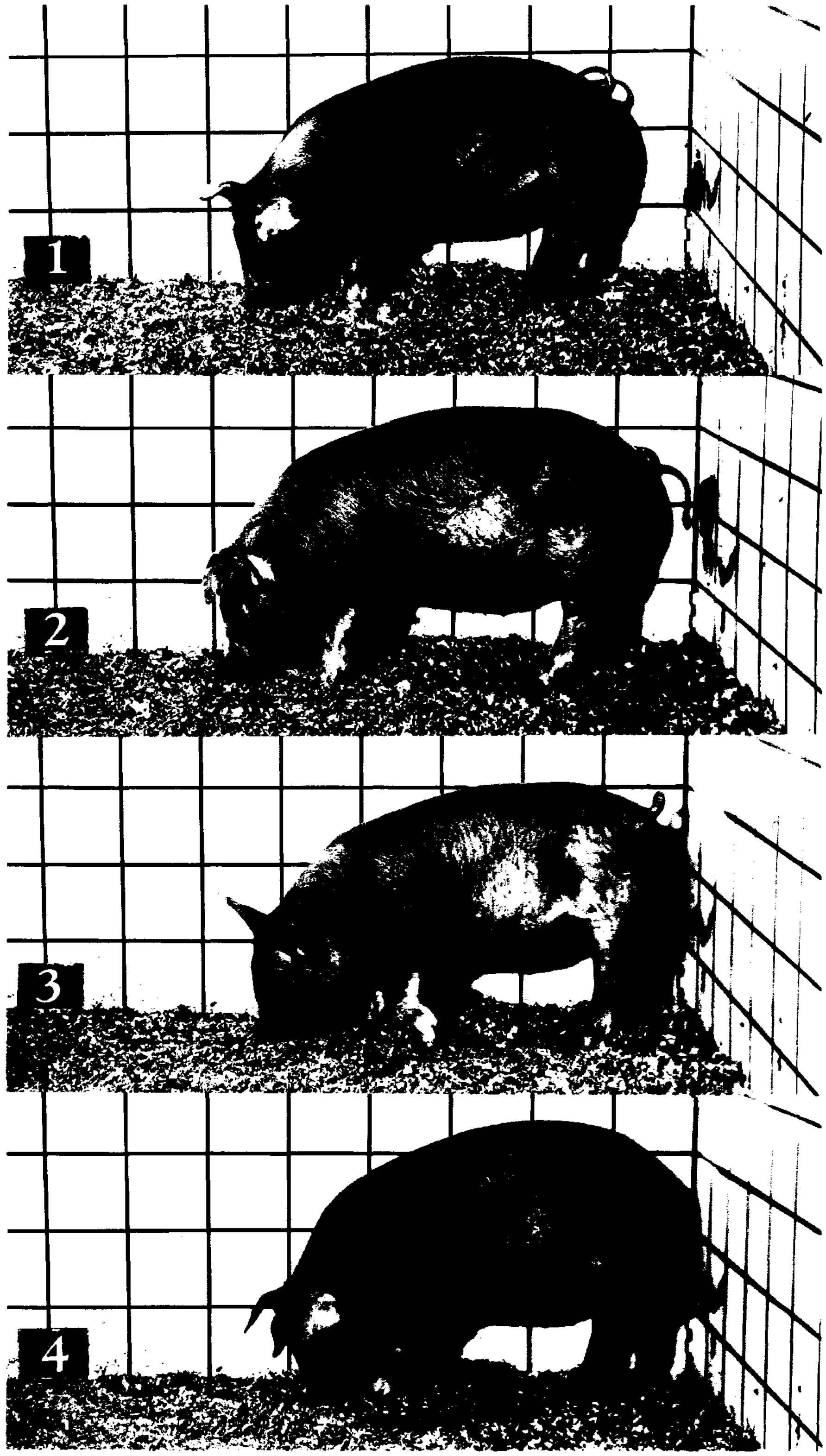

Figure 4. Litter 960 after 42 days on experiment

Figure 5. Litter 710 after 49 days on experiment

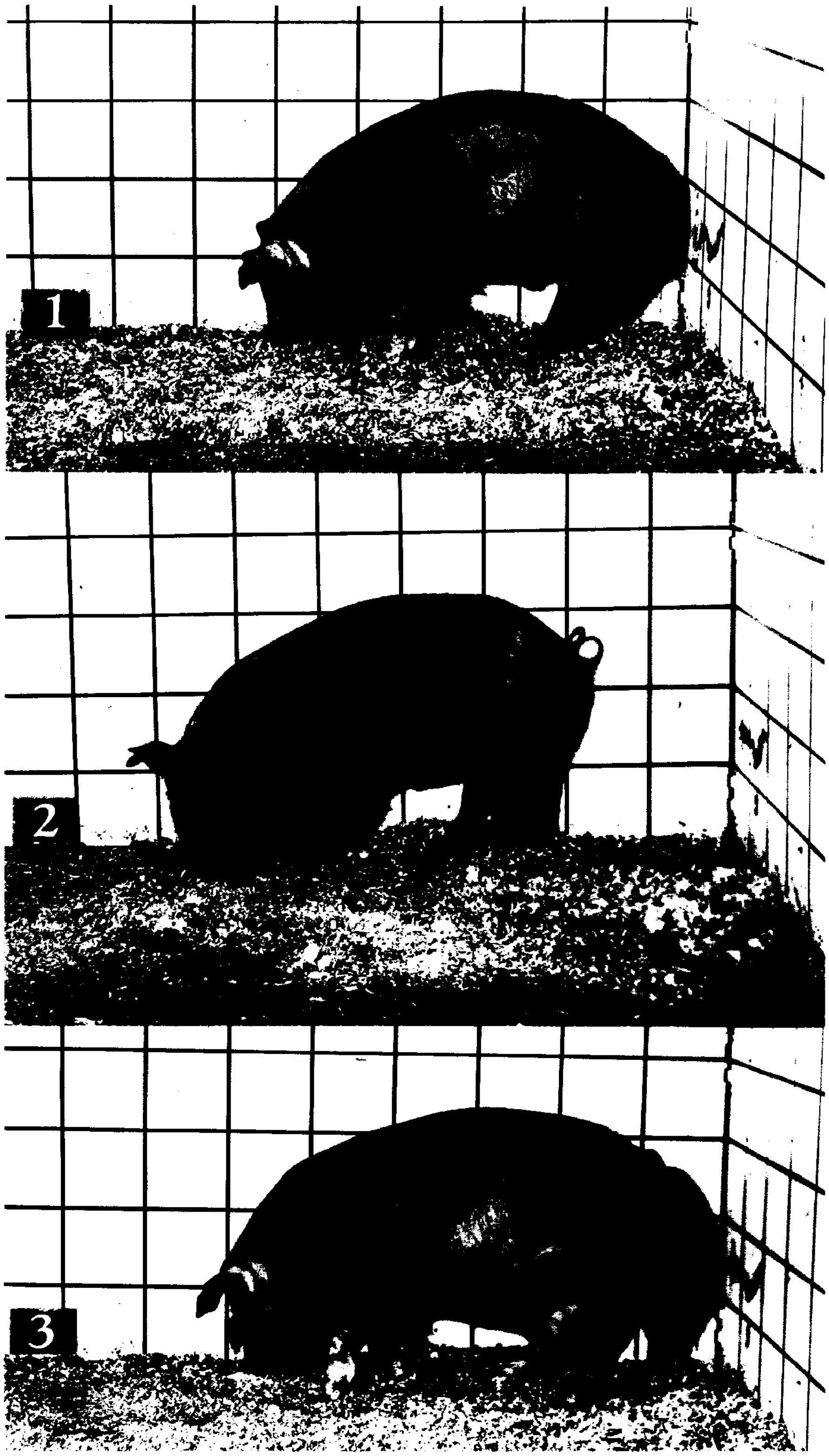

Figure 6. Litter 900 after 49 days on experiment

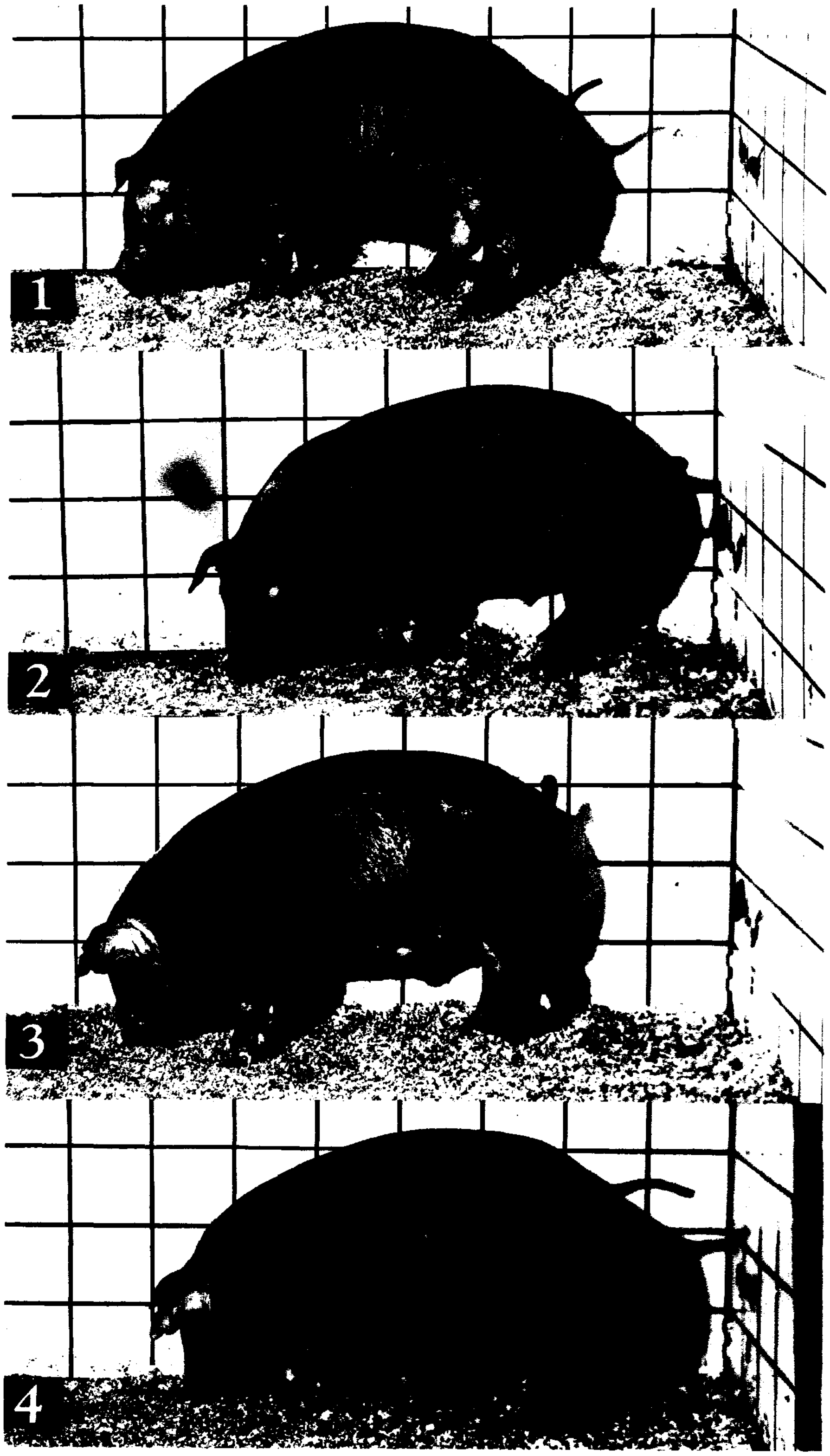

Figure 7. Litter 950 after 49 days on experiment

Figure 8. Litter 770 after 63 days on experiment

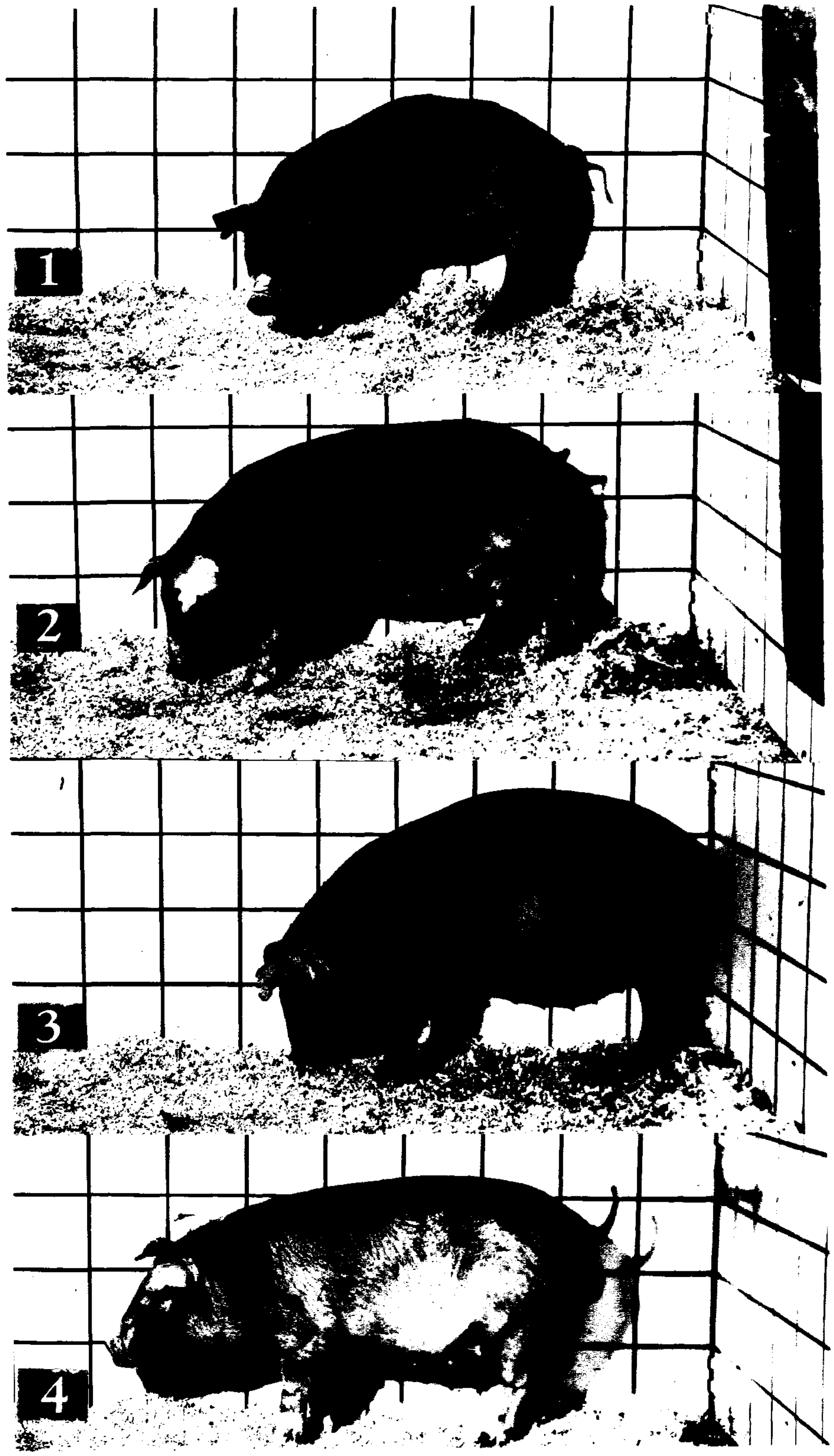

Figure 9. Litter 730 after 63 days on experiment

Table 14. Average daily gains (pounds) of individual pigs To 75 and 100 pounds live weight

	Micrograms of vitamin B_{12} per pound of ration							
	0		2		4		6	
	To 75	To 100	To 75	To 100	To 75	To 100	To 75	To 100
	(body weight lbs.)							
Litter 710B	1.114	0.831	1.175	0.923	1.333	1.413	1.736	1.548
" 730S	0.562	-	1.105	1.114	1.255	1.377	1.318	1.407
" 770S	1.018	0.976	1.089	1.147	1.333	1.397	1.173	1.211
" 900B	0.962	-	1.041	1.101	1.455	1.404	-	-
" 950B	1.297	1.043	1.536	1.511	1.500	1.489	1.533	1.365
" 960S	0.932	-	1.364	1.400	1.182	1.149	1.314	1.315
Average	0.981	-	1.218	1.199	1.343	1.372	1.410	1.369

daily gain to 75 pounds would be the same for pigs receiving 4 or 6 micrograms of vitamin B_{12} per pound of ration.

The original plan was to terminate the record of each pig as it reached 75 pounds live weight; however, the growth response and general appearance of the pigs indicated that more information could be obtained if the pigs were carried to a heavier weight. It was decided to obtain the daily gain and feed record to 75 pounds live weight and to keep the pigs on the experiment until they reached 100 pounds. A heavier weight was not possible because of the light gauge wire floor in the crates.

The pigs receiving 4 and 6 micrograms of vitamin B_{12} per pound of ration continued to gain rapidly. They required an average of 17.4 days to reach 100 pounds live weight after weighing 75 pounds. The average rate of gain for the two groups was the same. These pigs appeared normal in every way and did not show any deficiency symptoms. The pigs receiving 2 micrograms of vitamin B_{12} per pound of ration continued to gain. However, they required an average of 22.3 days to reach 100 pounds live weight after weighing 75 pounds. These pigs did not have the bloom or present the thrifty general appearance as did those receiving 4 and 6 micrograms of vitamin B_{12}, but none of them developed a clearcut deficiency symptom.

Three of the pigs on the basal ration reached 100 pounds live weight on an average of 34.3 days after weighing 75 pounds. They developed a rough skin and haircoat and presented a generally unthrifty appearance. The other three developed growth stasis and were very emaciated in appearance. One pig started to lose weight. Two of the pigs were injected intraperitoneally with 15 micrograms of vitamin B_{12} and 6 micrograms of vitamin B_{12} was added per pound of their ration. During the next 17 days these two pigs made gains of 20 and 27 pounds on 58 pounds of feed per pig. This is an average daily gain of 1.176 and 1.588 pounds as compared with 0.762 and 0.779 pound respectively before receiving vitamin B_{12}. Six micrograms of vitamin B_{12} per pound was added to the ration of another deficient pig. It gained 17 pounds in 12 days on 50 pounds of feed. This is an average daily gain of 1.417 pounds as compared with 0.562 before receiving vitamin B_{12}. In this case, feeding of vitamin B_{12} seems to have been just as effective as injection. These results prove, beyond a doubt, that vitamin B_{12} must be supplied to the growing pig if it is to make optimum growth.

The giving of vitamin B_{12} to the pigs on the basal ration prevents a statistical analysis of the results to 100 pounds live weight for all six pigs. However, an analysis of the three litters that did reach 100 pounds live weight shows the differences among rations to be significant at $P = 0.05$ as measured by the linear regression. Disregarding Lot I, an analysis of the pigs on the remaining treatments revealed no significant differences among vitamin B_{12} levels. The dramatic results obtained with injecting and feeding vitamin B_{12} to deficient pigs gives information of such value that it by far offsets any loss of information which could have been gained by statistical analysis.

Table 15 gives the average daily feed consumed and the feed required per 100 pounds gain to 75 and 100 pounds liveweight for the pigs fed different levels of vitamin B_{12}. There were no significant differences in the amount of feed consumed per day at either 75 or 100 pounds. There was a decrease in feed per hundred pounds gain up to 75 pounds liveweight as the vitamin B_{12} was increased. This was significant at the 5 per cent level as measured by linear regression.

As indicated earlier, the pigs used for this experiment were small, weak and anemic at the time of weaning. It was considered too risky to draw a large sample of blood for all analyses. However, a hemoglobin determination was made. No pig with a hemoglobin value of less than 3 grams per 100 milliliters of blood survived. Once the pig started eating, the hemoglobin level increased and recovery of the pigs was remarkable. It is interesting to note that the average hemoglobin value doubled from the beginning to the end of the experiment. A summary of the blood data is given in Table 16. There were no significant differences between ration treatments in the red and white blood cell counts. There was less total nitrogen in the blood of the basal pigs. There was a decided decline in urea as the amount of vitamin B_{12} fed was increased. This again indicates that vitamin B_{12} enhances the utilization of nitrogen.

Three pigs from each treatment were slaughtered after reaching 100 pounds liveweight. The average weights of the livers showed a downward trend as the vitamin B_{12} in the feed was increased; however, the amount

Table 15. Average daily feed consumed per pig and feed required per 100 pounds gain

	Micrograms of vitamin B_{12} per pound of ration							
	0		2		4		6	
	To 75	To 100	To 75	To 100 (body weight lbs.)	To 75	To 100	To 75	To 100
				Average daily feed (pounds)				
Litter 710B	3.200	3.273	2.975	2.821	3.267	3.696	3.739	3.952
" 730S	2.562	-	2.877	3.658	3.064	3.525	3.045	3.508
" 770S	2.857	3.119	2.750	3.187	3.262	3.638	2.673	2.972
" 900B	2.788	-	3.224	3.493	3.485	4.038	-	-
" 950B	3.405	3.371	4.321	4.556	4.179	4.667	3.967	4.442
" 960S	2.610	-	3.545	4.000	2.864	3.552	3.571	4.093
Average	2.904	-	3.275	3.619	3.354	3.853	3.399	3.973
				Feed per 100 pounds gain (pounds)				
Litter 710B	287.2	393.8	253.2	305.6	245.0	261.5	215.0	255.4
" 730S	455.6	-	260.3	328.4	244.1	256.0	231.0	249.4
" 770S	280.7	319.5	252.5	277.9	244.6	260.5	227.9	245.4
" 900B	290.0	-	309.8	317.1	239.6	287.7	-	-
" 950B	262.5	323.3	281.4	301.5	278.6	313.4	258.7	325.4
" 960S	260.0	-	260.0	285.7	242.3	309.1	271.7	311.3
Average	309.3	-	269.5	302.7	249.0	281.4	241.4	277.4

Table 16. Summary of blood data

Pig number	Hemoglobin grams per 100 ml. blood Start exp.	End exp.	Red blood cell count x 1,000	White blood cell count	Total nitrogen, grams per 100 ml.	Urea milligrams per cent
	Lot I. Basal ration					
774S	4.3	14.2	8050	18760	3.25	10.6
734S	4.6	12.9	6620	15120	3.01	13.2
953B	7.8	12.3	6520	14960	2.84	16.0
710B	9.5	14.1	6780	13960	2.88	10.5
901B	5.0	13.5	6610	11440	3.16	17.2
965S	5.5	12.7	8180	19000	3.32	13.7
Average	6.1	13.3	7127	15540	3.08	13.5
	Lot II. Basal ration + 2 micrograms vitamin B_{12} per pound ration					
778S	3.0	15.0	6660	23960	3.56	11.5
737S	3.5	12.0	7700	18440	3.08	11.6
950B	9.8	14.0	6920	16420	3.16	10.6
712B	7.5	12.5	7400	21440	3.05	9.7
943B	3.5	12.0	6260	27400	3.37	10.3
964S	7.5	16.0	4620	18160	3.18	10.9
Average	5.8	13.6	6593	21037	3.23	10.8
	Lot III. Basal ration + 4 micrograms vitamin B_{12} per pound ration					
776S	6.5	16.0	7400	22840	3.30	9.5
735S	3.6	11.2	6210	16520	3.06	12.2
951B	8.2	12.6	6990	11600	3.33	12.0
711B	7.8	13.3	6270	18360	3.25	9.7
900B	6.0	11.8	8660	20240	3.19	8.0
966S	5.5	14.8	9800	20600	3.24	10.8
Average	6.3	13.3	7388	18360	3.23	10.4

Table 16. (continued)

Pig number	Hemoglobin grams per 100 ml. blood Start exp.	End exp.	Red blood cell count x 1,000	White blood cell count	Total nitrogen, grams per 100 ml.	Urea, milligrams per cent
		Lot IV. Basal ration + 6 micrograms vitamin B_{12} per pound ration				
779S	4.5	13.7	6650	30360	3.14	9.0
736S	3.8	12.7	6320	25320	3.23	12.1
952B	7.6	12.9	6960	25160	3.22	7.7
713B	6.0	14.3	6760	19200	3.54	9.5
963S	8.7	12.3	8400	23600	3.18	9.3
Average	6.1	13.2	7018	24728	3.26	9.5

of vitamin B_{12} per gram of liver showed a decided increase. The average total amount of vitamin B_{12} in the livers of Lots III and IV is practically double that in Lots I and II. A summary of these data is shown in Table 17. The assay procedure of Kastelic (112) was used in determining these values.

A summary of the 24 hour collections of fecal and urinary volumes at the beginning and end of the experiment is shown in Table 18. Due to the depleted condition of the pigs, considerable difficulty was experienced in obtaining urine and fecal samples for assay at the beginning of the experiment. This may account for the large deviation in urine assay value of the pigs receiving 2 micrograms of vitamin B_{12} as compared with pigs on other levels of vitamin B_{12} as shown in Table 19. However, the vitamin B_{12} urinary value for these pigs is still out of line with the other groups at the end of the experiment. Assuming that pigs can synthesize vitamin B_{12}, there may be differences in the ability of individual pigs to do this. These results indicate that such may be the case.

A summary of the vitamin B_{12} assays on feces is shown in Table 20. Here again the vitamin B_{12} content of feces of pigs receiving 2 micrograms of vitamin B_{12} is seemingly out of line with the others at the beginning of the experiment. However, there is no great difference in the values per gram of feces at the end of the experiment. The total vitamin B_{12} values vary largely in proportion to the total feces voided.

Table 17. Vitamin B_{12} assay of livers

Lot	Pig	Liver weight in grams	Millimicrograms of vitamin B_{12} per gram	Total millimicrograms vitamin B_{12}
I	774	1021	80	81680
I	953	1163	32	37216
I	710	1277	36	45972
Average		1154	49.3	54623
II	737	1305	53	69165
II	950	1135	53	60155
II	712	709	84	59556
Average		1050	63.3	62959
III	735	1111	120	133,320
III	951	1078	110	118,580
III	711	851	100	85,100
Average		1013	110	112,333
IV	736	851	127	108,077
IV	952	823	160	131,680
IV	713	851	120	102,120
Average		842	135.6	113,959

Table 18. Volume of urine and weight of feces

Pig number	Micrograms vitamin B_{12} per pound ration	Start of experiment Milliliters urine	Start of experiment Grams feces	End of experiment Milliliters urine	End of experiment Grams feces
774	0	260	125	1570	620
734	0	155	85	1500	570
953	0	490	335	1760	390
710	0	590	330	1640	550
901	0	625	655	1730	265
965	0	560	245	1800	360
Average		447	296	1667	459
778	2	230	140	1650	900
737	2	175	105	4210	960
950	2	550	640	2640	1590
712	2	350	290	2660	480
943	2	410	360	3240	440
964	2	655	370	1930	1470
Average		395	317	2722	973
776	4	265	130	2360	1155
735	4	205	115	2430	2140
951	4	460	475	2710	1730
711	4	610	340	2900	1110
900	4	530	490	3670	1750
966	4	560	180	1950	1035
Average		438	288	2670	1487
779	6	235	140	2620	1010
736	6	220	130	2630	850
952	6	490	525	2260	880
713	6	525	250	3360	1040
963	6	550	200	2960	1445
Average		404	249	2766	1045

Table 19. Summary of vitamin B_{12} assays of urine

Micrograms vitamin B_{12} per pound of ration	Average milliliters urine in 24 hours	Millimicrograms of vitamin B_{12} per milliliter	Millimicrograms of vitamin B_{12} in 24 hours
	Beginning of experiment		
0	447	0.95	425
2	395	5.25	2074
4	438	1.05	460
6	404	1.00	404
	End of experiment		
0	1667	0.55	917
2	2722	1.90	5172
4	2670	1.05	2804
6	2766	1.05	2904

Table 20. Summary of vitamin B_{12} assays of feces

Micrograms vitamin B_{12} per pound of ration	Average grams of feces in 24 hours	Micrograms of vitamin B_{12} per gram	Micrograms of vitamin B_{12} in 24 hours
		Beginning of experiment	
0	296	0.105	32
2	317	0.30	95
4	288	0.125	36
6	249	0.30	75
		End of experiment	
0	459	0.92	422
2	973	0.85	827
4	1487	0.85	1264
6	1045	0.95	993

Summary

Six sets of four litter mates, three male and three female, were individually fed and watered *ad libitum* from weaning to 75 and 100 pounds liveweight in wire floored crates. The pigs were fed a fortified corn-soybean oil meal ration containing 20 milligrams each of aureomycin hydrochloride, terramycin hydrochloride, streptomycin sulfate and procaine penicillin G per pound of ration to which was added 0, 2, 4 and 6 micrograms of crystalline vitamin B_{12} per pound of ration. Litter mates were allotted at random to the various vitamin B_{12} levels.

The addition of vitamin B_{12} to the basal ration produced significantly greater gains to either 75 or 100 pounds liveweight. There was practically no difference in average daily gains of the pigs on the 4 or 6 microgram levels of vitamin B_{12}. Vitamin B_{12} deficient pigs on the basal ration exhibited a dramatic response to the intraperitoneal injection and oral feeding of vitamin B_{12}. Vitamin B_{12} also increased feed efficiency.

There were no significant differences in pigs fed different levels of vitamin B_{12} in hemoglobin, or red and white blood cell counts. The pigs fed the basal ration had the lowest level of total nitrogen and the highest level of urea in the blood. The amount of urea declined as the amount of vitamin B_{12} in the ration was increased. There was a tendency for the livers of the pigs to become lighter in weight as the

amount of vitamin B_{12} fed was increased; however, the amount of vitamin B_{12} per gram of liver and total vitamin B_{12} per liver increased. The urinary vitamin B_{12} values were not consistent except that the basal group was lower and the lot 2 group higher at both the beginning and end of the experiment. Differences in the vitamin B_{12} per gram of feces at the end of the experiment were small. The total amount varied with the total feces voided.

Pilot Experiment III.

Plan

The assay results in previous experiments consistently gave greater values for vitamin B_{12} in the feces and urine than could possibly be accounted for in the feed. This indicated that vitamin B_{12} was being synthesized in the digestive tract of the pig. Therefore, the purpose of this experiment was to determine the relative amounts of vitamin B_{12} present in food ingesta in certain sections of the digestive tract.

Twelve pigs, three from each level of vitamin B_{12}, were slaughtered from Experiment II. From about midway of the small and of the large intestine, sections were tied off and removed from each pig. The cecum was also tied off and removed. By this method of obtaining samples, the contents were kept in the gut and contamination was prevented. These sections containing the samples were immediately frozen at 10 degrees Fahrenheit and held at that temperature until assayed. In order to make the results more comparable, the same assay procedure was used as that used in assaying the feces.

Results and discussion

Table 21 gives a summary of the vitamin B_{12} assays obtained on samples taken from the small intestine, cecum and large intestine. The vitamin B_{12} values for the small intestine show an increase as the

Table 21. Summary of assays of digestive tract contents

Lot	Pig number	Millimicrograms vitamin B_{12} per gram			
		Small intestine	Cecum	Large intestine	Lot average per gram of feces
I	710	19	420	730	
I	774	9	460	1050	
I	953	8	340	900	
Average		12	407	893	920
II	712	48	360	1600	
II	737	6	110	500	
II	950	17	240	400	
Average		23.7	237	833	850
III	711	21	280	470	
III	735	42	260	370	
III	951	19	320	325	
Average		27.3	287	388	850
IV	713	52	360	1050	
IV	736	40	240	530	
IV	952	10	280	770	
Average		34	293	783	950
Av. all pigs		24.3	306	808	892

amount of vitamin B_{12} fed is increased. The vitamin B_{12} added in the feed is probably responsible for these differences. There is a very great increase in the vitamin B_{12} values obtained for the cecum, a fairly great increase in the large intestine over the cecum and a slight increase in the feces over the large intestine indicating synthesis of vitamin B_{12}. Considerable variation was encountered with individual pigs; however, the general trend is the same in all cases. Probably the average assay values for all pigs presents the best overall picture of what is happening in the digestive tract. There is a tendency toward higher vitamin B_{12} values in the cecum and large intestine of the basal ration group. It is believed that this was an attempt on the part of the intestinal microflora to overcome the vitamin B_{12} deficiency of the ration. Partial constipation and slower movement through the digestive tract, thus allowing more time for bacterial action, could have also aided in bringing about these higher vitamin B_{12} values for the intestinal contents of the pigs fed the basal ration.

Paper strip chromatography indicated that the substance being measured did not move on the paper strip. Alkaline hydrolysis in approximately .2 N potassium hydroxide destroyed 5 to 50 per cent of the activity of the small intestine samples and gave essentially complete destruction of activity in the cecum and large intestine samples. Different assay levels in the small intestine samples gave a drift in assay values characteristic of compounds other than vitamin B_{12} which stimulate the test organism. The alkaline stable material in the small intestine samples probably account for the drift in assay values. Different levels of cecum and large intestine samples gave consistent assay values indicating only vitamin B_{12} activity was being measured.

It is not intended that the above results and discussion be taken as complete evidence as to what happens in the digestive tract of the pig with regard to vitamin B_{12}. However, it is believed that this type of study can yield fruitful information. This work is only the beginning of much more work that could and should be done. Even though the assay results are reported as vitamin B_{12} in these experiments, the reader should bear in mind that it might in all cases be more correct to refer to these values in terms of response of the *Lactobacillus leichmanii* organism.

Summary

A section of small intestine, cecum and large intestine including their contents was obtained from twelve pigs at the termination of Experiment II. Three pigs were from each of four lots receiving 0, 2, 4 and 6 micrograms of vitamin B_{12} per pound of ration. The small intestine contents gave an increase in vitamin B_{12} as the amount fed was increased. There was considerable variation between pigs. However, the general trend showed an increase in the amount of vitamin B_{12} present in the intestinal contents from the upper to the lower part of the digestive tract. The greatest increase was in the cecum. There was also a considerable increase in the large intestine. These results indicate that vitamin B_{12} or vitamin B_{12} like substance(s) is synthesized by the microflora in the lower part of the digestive tract of the pig.

GENERAL DISCUSSION

The purpose of this study was to determine the nutritional requirement of vitamin B_{12} for the weanling pig. The nutritional requirement as used here may be defined as that amount of vitamin B_{12} which must be present in the ration of the animal to permit optimum growth. This requirement should not be confused with the total requirement of the animal which is supplied by the ration and synthesis within the animal. The reader is again reminded of the fact that crystalline vitamin B_{12} was used throughout this study.

The feeding of vitamin B_{12} without antibiotics in Experiment I gave small increases in daily gain and feed efficiency as the amount of vitamin B_{12} was increased in the ration. Based upon these results, it appears that much higher levels of vitamin B_{12} would have to be fed to satisfy the nutritional requirement. When a combination of equal parts of aureomycin hydrochloride, procaine penicillin G, terramycin hydrochloride and streptomycin sulfate was fed at the rate of 40 milligrams per pound of ration to control the microflora of the intestines, the addition of crystalline vitamin B_{12} at levels of 5, 10 and 20 micrograms per pound of ration gave equal response in weight gains and feed efficiency above that obtained on the basal ration alone. Therefore, it was evident that the nutritional requirement of vitamin B_{12} was 5 micrograms or less per pound of ration when fed in the presence of large amounts of a combination of antibiotics to control intestinal microflora.

One pilot experiment further indicated that there is a storage of vitamin B_{12} in the pig and the length of carryover was observed to be from 3 to 8 weeks. Another pilot experiment indicated that an increased amount of antibiotics might affect a more complete control of the intestinal microflora. In the second experiment, the same basal ration was used as in Experiment I except that ascorbic acid was added and the amount of each antibiotic was doubled to give a total of 80 milligrams per pound of ration. To this basal ration was added 0, 2, 4, and 6 micrograms of crystalline vitamin B_{12} per pound. The results show that the 4 and 6 microgram levels of vitamin B_{12} gave the same results both from the standpoint of gain in weight and feed efficiency. Therefore, it was concluded that the optimum nutritional requirement of the weanling pig is 4 micrograms of vitamin B_{12} per pound of a corn-soybean oil meal ration when a combination of antibiotics is fed to control the microflora of the intestinal tract. This is in close agreement with the requirement of 5 micrograms of vitamin B_{12} per pound of ration as found by Lepley (119) and Vohs (218) for growing-fattening pigs. Assuming that the need of the nursing pig is higher, these results are also in line with those obtained by Nesheim et al. (140, 141) and Anderson and Hogan (7).

One may ask whether or not vitamin B_{12} is synthesized by the pig. The question also arises regarding the effect of the antibiotics in controlling the microflora of the intestinal tract. These are questions that cannot be definitely answered, because, in the first place, no one knows the normal microflora of the pig's digestive tract. The microflora no doubt also vary with the composition of the ration being fed. However, there are certain facts which should be pointed out. Pigs on the basal ration which contained antibiotics developed vitamin B_{12} deficiencies, whereas, those on the basal ration without antibiotics in Experiment I did not. This seems to indicate that the intestinal microflora of the pig under normal conditions can synthesize enough vitamin B_{12} to permit slow growth even when the ration is deficient in vitamin B_{12}.

The antibiotics also controlled the organisms which utilize vitamin B_{12} and subsequently compete with the pig for the vitamin B_{12} present in the digestive tract. This is clearly demonstrated by the fact that very small quantities of vitamin B_{12} in the presence of antibiotics produced just as good or better gains in weight and feed efficiency than larger amounts of vitamin B_{12} without antibiotics. In the presence of antibiotics, the average daily gain for the 2 micrograms level of vitamin B_{12} was 1.22 pounds and 4 micrograms produced 1.34 pounds as compared with 1.29 pounds daily gain on 20 micrograms of vitamin B_{12} without antibiotics.

The dramatic recovery obtained by injection and feeding of vitamin B_{12} to the deficient pigs proves that vitamin B_{12} is essential for the growing pig.

The addition of vitamin B_{12} did not produce any significant changes in hemoglobin values which is in agreement with the findings of Beeson et al. (18) and Bowland et al. (35). There were no significant differences in the red and white blood cell counts. All were within the normal range.

In general, the blood total nitrogen was lowest in the pigs fed the basal ration. This is particularly evident in Experiment II. It is also obvious that there is a decrease in the amount of urea in the blood as the amount of vitamin B_{12} fed is increased. Apparently vitamin B_{12} is functioning in the absorption of nitrogen or amino acids as well as aiding in the utilization of the amino acids by the tissues. These results agree with findings of other workers. Abbot (1) found that rabbits injected with 5 micrograms of vitamin B_{12} daily showed marked positive nitrogen balance. Urinary excretion of nitrogen, urea and amino acids was diminished. When administration of vitamin B_{12} was stopped, the nitrogen balance returned to its former level. Charkey et al. (51) observed that vitamin B_{12} lowered the non-protein nitrogen in chick blood and apparently enhanced utilization of circulating amino acids. Stern and McGinnis (207) also observed a higher percentage of non-protein nitrogen in the blood of deficient chicks than in chicks injected with vitamin B_{12}. Oginsky (152) found that liver homogenate of rats fed vitamin B_{12} formed methionine from homocystine faster than liver homogenate from vitamin B_{12} deficient rats.

The livers showed a definite trend toward becoming lighter in weight as the amount of vitamin B_{12} fed was increased; however, the assay values and total vitamin B_{12} per liver increased. These findings are interesting but no explanation of the results is known at present.

The most intriguing part of the urinary and fecal assays is that they consistently showed greater vitamin B_{12} values than could possibly be accounted for in the ration. This definitely pointed to synthesis of vitamin B_{12} in the pigs' digestive tract. A pilot experiment, in which ingesta from the small intestine, cecum and large intestine were assayed, indicated that vitamin B_{12} or vitamin B_{12} like substance(s) is synthesized in the lower part of the pig's digestive tract.

SUMMARY

The experiments reported herein were conducted to determine the amount of vitamin B_{12} which must be supplied in the ration to obtain optimum growth with the weanling pig. The pigs were individually fed in wire floored crates.

The addition of vitamin B_{12} to a fortified corn-soybean oil meal ration produced a slight increase in average daily gain as 0, 5, 10 and 20 micrograms were fed per pound of ration. With the addition of 10 milligrams each of aureomycin hydrochloride, terramycin hydrochloride, streptomycin sulfate and procaine penicillin G, vitamin B_{12} gave a much greater response in weight gains but there were no differences between the added levels of vitamin B_{12}. Further study, using 0, 2, 4 and 6 micrograms of vitamin B_{12} per pound of a fortified corn-soybean oil meal ration in the presence of 20 milligrams each of the same four antibiotics, showed that 4 micrograms produced practically the same performance as 6 micrograms of vitamin B_{12}. Pigs which developed vitamin B_{12} deficiencies showed excellent recovery to the injection and feeding of vitamin B_{12}. Feed efficiency, especially in the presence of antibiotics, was improved by the addition of vitamin B_{12}.

There were no differences among the pigs fed different levels of vitamin B_{12} with respect to hemoglobin, red or white blood cell counts. Total nitrogen in the blood showed some variation but there were no

significant differences among ration treatments. However, there was a decline in blood urea as the amount of vitamin B_{12} was increased in the ration. As the amount of vitamin B_{12} fed was increased, there was a tendency for the total weight of the livers to become lighter; however, the total amount of vitamin B_{12} per liver was increased. It appears that 10 micrograms or less of vitamin B_{12} per pound of ration allow maximum vitamin B_{12} storage in the liver. There was a variation in the individual pig liver storage of vitamin B_{12}. Therefore, pigs placed on a vitamin B_{12} deficient diet will vary in the length of time needed to show signs of vitamin B_{12} depletion and subsequent deficiency. The time varied from about 3 to 8 weeks in these experiments for vitamin B_{12} depletion to become evident.

Assays of the urine and feces showed the presence of larger amounts of vitamin B_{12} than could be accounted for in the ration consumed. Assay of the contents of digestive tracts indicated synthesis of vitamin B_{12} or vitamin B_{12}-like substance(s) in the lower part of the pig's digestive tract. There was considerable variation in this respect among individual pigs.

CONCLUSIONS

The results of this study warrant the following conclusions:

1. The weanling pig has a nutritional requirement for vitamin B_{12}.
2. The nutritional requirement of the weanling pig for vitamin B_{12} for efficient gains was found to be 4 micrograms or less per pound of total ration when fed a corn-soybean oil meal ration with a combination of antibiotics for partial control of intestinal microflora.
3. The nutritional requirement of vitamin B_{12} is less when antibiotics are fed.
4. Feeding antibiotics inhibits intestinal microflora which compete with the pig for vitamin B_{12}.
5. Feeding antibiotics also inhibits intestinal microflora which synthesizes vitamin B_{12}.
6. The feeding of vitamin B_{12} does not cause any significant differences in hemoglobin or red and white blood cell counts.
7. There were no significant differences in total nitrogen in the blood; however, there was a tendency for the amount to be less when vitamin B_{12} was not fed.
8. Feeding vitamin B_{12} reduced blood urea levels.
9. There was a tendency for liver weights to decrease as the amount of vitamin B_{12} fed was increased; however, the total vitamin B_{12} in the liver increased.

10. There is sufficient storage of vitamin B_{12} in the pig for effective carry-over effects for about 3 to 8 weeks.

11. A corn-soybean oil meal ration with a combination of antibiotics can be used to produce vitamin B_{12} deficient pigs.

12. There is synthesis of vitamin B_{12} or vitamin B_{12}-like substance(s) in the lower part of the pig's digestive tract.

The following are suggestions for further study:

1. It is suggested that a mixture of antibiotics similar to the ones used here might be used in studying the minimum requirement of different vitamins which may be synthesized in the intestinal tract of the pig. The basal ration should be void of or contain insignificant amounts of the vitamin being studied.
2. In future studies to further evaluate the usefulness of antibiotics in feeding swine, pathological and histological studies of tissues and organs should be made to determine the effect of antibiotics. Weights, measurements, and other information which may seem pertinent should be obtained.
3. More information about the possible synthesis of vitamin B_{12} in the digestive tract of the pig is needed. First of all, more work needs to be done on the method of vitamin B_{12} assay. It appears that each biological substance has its own peculiarities which must be coped with before a satisfactory assay can be made. Individual pigs of different ages from weaning to market weight should be used in a study of this kind. It is believed that younger pigs have a greater requirement for vitamin B_{12} and at this time it appears that their intestinal microflora are least capable of synthesizing vitamin B_{12}. Healthy, normal growing pigs fed rations with and without different antibiotics and in different combinations should be studied.

Food ingesta samples taken from the same section of the digestive tract will vary in their vitamin B_{12} assay value. To overcome this, it is suggested that the contents for assay, from each individual section to be studied, be emptied at the time of slaughter and blended. By doing this, a more uniform sample for assay could be obtained from each section of the digestive tract.

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